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Proceedings of the 7th Meeting of IUFRO Working Party 7.03.04 Diseases and Insects in Forest Nurseries



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Proceedings of the Seventh Meeting of IUFRO Working Party 7.03.04

Diseases and Insects in Forest Nurseries

Hilo, Hawai'i USA
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Edited by
Michelle M. Cram

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First Row: Yvonne Barkley, Susan Schenck, Scott Enebak, Nancy Gillette, Elsie Burbano, Ayami Shiraishi, Sri Rahayu, Nitin Kulkarni, Bohun Kinloch.

Back Row: Audrius Menkis, Anna Rytkonen, Kerry Britton, Jerry Weiland, Arja Lilja, Pamala Reeves, Robert (Bob) James, Shaily Menon, Phil Cannon, Michelle Cram.

Participants not included in photo: Nick Dudley, Aileen Yeh, Tyler Jones, Michael Kaufmann , Patrick Conant, Lloyd Loope, Anne Marie La Rosa, Scot Nelson, Chris Kadooka.

(Photo by Stephen Fraedrich, USDA Forest Service, Southern Research Station)

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PREFACE

The 7th meeting of IUFRO Working Party 7.03.04 was held July 12-17 in Hilo, Hawai'i, USA. Non-native diseases and insects and their implications for forest nurseries and native flora were the special focus of this meeting. Several speakers that work on non-native pests in Hawai'i gave presentation on diseases and insects that have had a major impact on the native forest trees and ecosystems. Field trips to State, University and the National Park nurseries highlighted the challenges of growing native plants for reintroduction and rehabilitation of Hawaii's unique ecosystems. Two of the most serious pathogens affecting native trees are *Fusarium oxysporum* causing koa wilt, and *Puccinia psidii* because of potential effects on 'ōhi'a, a dominant native tree. During field trips, participants were able to view 'ōhi'a trees and koa wilt. A visit to the Maunawilli research site of the Hawai'i Agriculture Research Company allowed participants to see koa wilt resistance testing; as well as outplantings of resistant koa trees.

Participants from other countries also presented on non-native diseases affecting nurseries. In Indonesia, a gall rust caused by *Uromycladium tepperianum* has been identified since 1993 on batai causing severe damage to all growth stages of the plant, particularly on seedlings in the nursery. Participants from Finland reported recent introductions of non-native pests affecting tree production including *Phytophthora cactorum*, as well as, ascomycetes *Mycosphaerella pini* (anamorph: *Dothistroma septosporum*) and *Hymenoscyphus albidus* (anamorph: *Chalara fraxinea*). The Sudden Oak Death pathogen, *P. ramorum*, has been found annually by Finnish Food Safety Authority in imported plants as well as in two domestic nurseries producing horticultural plants.

Internationally there is an effort to monitor for pests of expatriate plants. Information gathered from monitoring could be used by capitalizing on a sentinel plant network in botanical gardens and arboreta and monitoring pests in foreign environments. Several presenters advocate more strict regulations on nursery plant movement between countries and states. One example is the need to reemploy strong importation regulations to permanently protect both native and introduced Myrtaceae.

Other papers presented research findings on a wide variety of disease and insect problems and their control from India, Lithuania, Indonesia, Finland, and the USA. Many of the participants were interested in the results of testing Proline 480 SC® (41% prothioconazole) against diseases of southern pine nurseries in the USA and the possible use of this new fungicide on other disease problems.

Our working party has accepted the offer by Rimvydas Vasaitis and Audrius Menkis from Sweden to have our 8th meeting in Palanga, Lithuania. Our new coordinator is Dr. Rimvydas Vasaitis. His two deputies will be Dr. Audrius Menkis and Ms. Michelle Cram. We look forward to our meeting in Lithuania.

--Michelle Cram, Coordinator for the 7th meeting of WP7.03.04.

PROTECTING HAWAII'S FORESTS FROM HARM: AN ARGUMENT FOR STRONG MEASURES TO PREVENT ARRIVAL OF PESTS OF HAWAII'S MYRTLE FAMILY

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ABSTRACT

The Neotropical rust fungus *Puccinia psidii*, notorious for the severity of its impacts and its broad host range in the family Myrtaceae, is believed to pose the greatest current threat to Hawaii's forests, including *Eucalyptus* plantations. Multiple strains of this rust have demonstrated ability to infect different suites of host plants. Only one genotype established in Hawai'i in early 2005 and has not mutated. The addition of new genetic material of *P. psidii* would increase the likelihood that more species in the Myrtaceae will be seriously affected, most notably Hawaii's dominant native forest tree 'ōhi'a, *Metrosideros polymorpha*, which is only moderately damaged by the strain of rust currently in Hawai'i. The potential consequences of a virulent strain of rust on 'ōhi'a forests are immense, due to its role as a foundation tree species and the diversity of niches it fills in Hawai'i. Currently, the State of Hawai'i regulates incoming plant material in the family Myrtaceae by visual inspection, but inspection capacity and latent infections seriously limit the ability to detect the rust. Only complete or near-complete interdiction of Myrtaceae would seem to offer strong protection for Hawaii's native ecosystems. Prohibition or restriction of the flow of Myrtaceae into Hawai'i could also reduce establishment of numerous invasive insects and pathogens of Myrtaceae, notably for 90 species of non-native *Eucalyptus*, a potentially important forest resource for Hawaii's future.

INTRODUCTION

The Hawaiian Islands are justifiably famous for their biological uniqueness but have lost roughly half of their original native-dominated habitat (Loope 1998). Dry and mesic forests have been reduced most drastically, whereas wet forest remains relatively intact. Hundreds of species have been lost to extinction, including at least 106 of 1300 plant taxa, three-fourths of ca. 1000 endemic snail species, and at least 83 of 115 endemic land bird species. With 0.4% of the land area of the USA, Hawai'i harbors over 25% of the country's federally listed Endangered species. While habitat destruction by humans has been a direct factor in Hawaii's ecological losses in the past, human-facilitated biological invaders, including forest insects and pathogens, are currently the primary agents of continuing degradation.

A single tree species, 'ōhi'a (*Metrosideros polymorpha*), in the myrtle family (Myrtaceae), is the keystone species of Hawaii's surviving native forest; about 400,000 ha remain. 'Ōhi'a inhabits a broad range of sites, from sea level to 2500 m elevation, and dominates extensive

areas of Hawaii's important watersheds. Forest pests that might threaten 'ōhi'a forest are therefore of special concern. An important network of "Watershed Partnerships" has evolved over the past two decades to assist with management of much of this land (<http://hawp.org/partnerships.asp>). These partnerships have multiple goals of watershed and biodiversity protection. The major threats to be addressed by the partnerships include feral pigs and aggressively invasive plant species, but the potentially overwhelming importance of forest pests was signaled in April, 2005, by detection of the rust fungus *Puccinia psidii* on an 'ōhi'a sapling on O'ahu and the pathogen's subsequent statewide spread. This pathogen is notorious for its extremely broad host range within the myrtle family and very severe effects on certain species (Coutinho and others 1998).

At least 15 genera and 49 species of Myrtaceae are grown in Hawaii's "gardens" as ornamentals, fruit crops, shade and street trees; additional genera and species can be found in botanical gardens (Staples and Herbst 2005). Species of the myrtle family (Myrtaceae) are numerically important in the flora and dominate the vegetation over extensive areas of Hawai'i. There are eight native species of Myrtaceae and nearly 200 non-native species among the cultivated and naturalized flora (Imada and others 2006). Nearly half the introduced species of Myrtaceae in Hawai'i are species of *Eucalyptus*, almost all native to Australia (*E. deglupta* and *E. urophylla* are native to Philippines, Indonesia, and Timor.)

Very significant plantings of non-native *Eucalyptus* species were made in Hawai'i, primarily on degraded lands of the five main Hawaiian islands, with most planting activity in the 1930s and 1960s. Frequently, they are single-species plantings of *Eucalyptus robusta*, a tree with thick reddish brown bark; more than 2.3 million trees were planted by the Hawai'i Division of Forestry before 1960 and similar numbers were planted by private landowners (Little and Skolmen 1989). *E. saligna*, a tree with smooth bluish gray bark, is the only species that rivals *E. robusta* in quantity; 437,000 trees were planted prior to 1960 and one million trees after 1960 (Little and Skolmen 1989). Along highways at low elevation the most common other species seen are *E. citriodora* (now included in *Corymbia*), with gray dimpled bark, and *E. deglupta*, with pink and green scaly bark. At higher elevations, the common species are *E. sideroxylon*, with black bark, and *E. camaldulensis*, with bark mottled gray and brown. Hawai'i has more than 90 species of *Eucalyptus* as well as many closely related Australian Myrtaceae, and they are often found in mixed plantings. Additional common *Eucalyptus* spp. include: *E. botryoides*, *E. globulus*, *E. grandis*, *E. microcorys*, *E. paniculata*, *E. pilularis*, and *E. resinifera* (Little and Skolmen 1989).

During the 1980s, a research project was conducted in the Hāmākua and Ka'u districts of the Big Island that developed guidelines for establishing and managing short-rotation (5 to 8 years) *Eucalyptus* plantations in Hawai'i and explored whether woody biomass is a suitable source of bioenergy (Whitesell and others 1992). *Eucalyptus grandis* and *E. saligna* were found to be the best performers. Provenances, specific areas where seed should be obtained, were identified in Australia for those two species (Skolmen 1986).

In the decade after 1996, more than 20,000 acres of industrial eucalyptus plantations were established in Hamakua, Ka'u, and Waimea. Most of these were pure stands of *E. grandis*. An additional 2,000 acres of mixed *E. deglupta* and *Falcataria moluccana* stands have been

established on Kaua'i. These plantations are intended for commercial harvest, either for solid wood products or bioenergy (J.B. Friday, pers. comm.).

Based on its effects in Brazilian plantations over several decades, *Puccinia psidii* is considered to be the most serious threat to eucalypt plantations worldwide (Coutinho and others 1998), but the strain already in Hawaii has not been observed to be problematic for *Eucalyptus*.

The jury is still out on the proper role of *Eucalyptus* in Hawaii, but options for its cultivation should be kept open. *Eucalyptus* has been out of favor, some would say neglected, as a resource for the past quarter century. *Eucalyptus* spp. in Hawaii, having not yet accumulated a serious array of forest pests, may provide an important natural resource for the future -- lumber, veneer, specialty woods, biofuels, and perhaps decorative foliage. The most serious eucalypt insect pests to date are discussed in Conant and others (this volume); their impacts have so far been modest. *Cryphonectria cubensis*, an internationally widespread canker disease, has been the most serious plant pathogen of *Eucalyptus* in Hawaii since the 1980s (Whitesell and others 1992), though *Coniothyrium zuluense*, another canker disease, an apparent recent introduction from South Africa, may also have serious potential for damage (Cortinas and others 2004). This latter pathogen is most likely to have been transported from South Africa to Hawaii in *Eucalyptus* seed (M.J. Wingfield, pers. comm., 2007) It is clear that there needs to be careful coordination between Hawaii's Department of Agriculture's Plant Quarantine Branch and Hawaii's incipient *Eucalyptus* industry.

Protecting Hawaii's forests from invasive pests is particularly challenging and requires well thought out measures. Hawaii's susceptibility to invasions is dramatically illustrated by the fact that nearly as many non-native arthropod species are established in Hawaii as in the other 49 states of the USA. How can this be possible? A consultant's report to the U.S. Department of Agriculture (McGregor 1973) recognized that innate characteristics of Hawaii seem to make agricultural quarantine more difficult: "[For insects and mites] in the period 1942-72 the rate of colonization per thousand square miles was 40 species, 500 times the rate of continental United States..." in spite of a larger quarantine force in relation to volume of commerce. He speculated on possible reasons: "Although there is much greater diversity of crops and habitats within the continental United States, these are dispersed over a vastly larger land area. In Hawaii...the various habitats are [compressed and] more readily accessible from the principal port of entry. The more moderate and stable climate is also more favorable for an invading species than is the climate over much of the United States."

THE RUST FUNGUS *Puccinia psidii* AND ITS IMPLICATIONS FOR HAWAII.

The Neotropical rust fungus *Puccinia psidii* was originally known primarily from the host tree common guava (*Psidium guajava*) in its native Brazil, but has been found since on hosts throughout the myrtle family (Myrtaceae), including a dramatic host jump to non-native eucalypt plantations (Coutinho and others 1998, Glen and others 2007). Most rust fungi are able to live only on a very narrow range of host species. *P. psidii* is unusual both for having a broad host range and for the intensity of its damage to susceptible young growth. This rust

first got a foothold in the USA in Florida over three decades ago. The federal Department of Agriculture (USDA) has since considered it a non-actionable, non-reportable pest. Hawai'i and Florida are the only two states with native species in the myrtle family. Over 30 years this rust has done little damage to any of the scattered native Myrtaceae in Florida, though the host range of the rust has gradually grown to about 30 mostly non-native species in the family, apparently because of increasing genetic variety of the rust by repeated introductions. However, Florida's native Myrtaceae are among the roughly 1,100 Neotropical species that are largely resistant to *P. psidii*. The 3,000 species of Paleotropical Myrtaceae (of the Pacific, Australia, Asia and Africa) are expected to prove much more vulnerable to *P. psidii*, because they lack evolutionary exposure to the pathogen for many millions of years (Simpson and others 2006). Little is known about the genetics or genetic strains of *P. psidii*, though there are apparently numerous strains that have differential ability to infect suites of host plants (Glen and others 2007).

The rust was first recorded in the state of Hawai'i on O'ahu in April 2005 (Killgore and Heu 2005) and quickly spread throughout the Hawaiian Islands. The main concern in Hawai'i became the potential threat to 'ōhi'a, *Metrosideros polymorpha* (Myrtaceae). The potential ecological consequences of a strain of the rust highly virulent to 'ōhi'a are immense, due to its role as a foundation tree species and the diversity of niches it fills in Hawai'i.

A single genetic strain of the rust is established in Hawai'i, apparently composed of a single genotype lacking successful sexual reproduction (S. Zhong, J. Uchida, and C. Kadooka, pers. comm.). *Puccinia psidii* has been found in Hawai'i statewide attacking Myrtaceae from near sea level to about 1,200 m elevation in areas with rainfall ranging from 750–5,000 mm (R. Anderson & J.B. Friday, pers. comm.). Five of eight native Myrtaceae and at least 15 non-native species have been observed as hosts of *P. psidii* in Hawai'i (Table 1). The federally Endangered and endemic *Eugenia koolauensis* (*nioi*) and the non-Endangered indigenous species *Eugenia reinwardtiana* are severely damaged. The introduced and invasive rose apple, *Syzygium jambos*, is severely affected at a landscape scale, with widespread crown dieback and many instances of complete tree death. In spite of billions of wind-dispersed rust spores produced from rose apple infestations during 2006 to 2008, adjacent 'ōhi'a have been little affected to date by the rust strain in Hawai'i. Within the elevation range of the rust, *P. psidii* is found on less than 5 percent of the 'ōhi'a trees; of those it is normally found on less than 5 percent of the leaves (R. Anderson, pers. comm.).

The rust strain in Hawai'i does not utilize many of the species known to be infected by the rust elsewhere, including common guava (Coutinho and others 1998, Glen and others 2007). Based on the substantial genetic diversity of the crop-damaging species of the genus *Puccinia* (e.g., Steele and others 2001, Chen 2005, Broeker and others 2006), there is good reason to believe that there are dozens and possibly hundreds or thousands of genotypes of *P. psidii*. Although these genotypes are likely concentrated in the core range in Brazil, there is the potential for dispersal by globalization. Multiple genotypes are believed already present in the USA (Zhong and others 2008, S. Zhong, pers. comm.) and certain to spread freely in the absence of restrictions. The USDA Forest Service has initiated a major collaborative project in Brazil to investigate the genetics of susceptibility of Hawai'i's 'ōhi'a to *P. psidii* (Cannon and others 2009), but initial results will likely not be available for at least several years. If

Table 1. Hosts and damage levels of the Neotropical rust fungus *Puccinia psidii* in Hawai'i, 2005-2009, among selected Myrtaceae species (not comprehensive).¹

Latin name	Common name	Native to	Level of damage
<i>Chamelaucium uncinatum</i>	Geraldton waxflower	W. Australia	moderate
<i>Eucalyptus</i> spp.	eucalypts	Australia & vicinity	tolerant ²
<i>Eugenia koolauensis</i>	nioi	endemic to Hawai'i	severe
<i>Eugenia reinwardtiana</i>	nioi	indigenous to Hawai'i	severe
<i>Eugenia uniflora</i>	Surinam cherry	Neotropics	light
<i>Melaleuca quinquenervia</i>	paperbark	Australia & vicinity	moderate
<i>Metrosideros polymorpha</i>	'ōhi'a	endemic to Hawai'i	light
<i>Metrosideros tremuloides</i>	'ōhi'a	endemic to Hawai'i	light
<i>Myrtus communis</i>	myrtle	Mediterranean region	moderate
<i>Pimenta dioica</i>	allspice	Neotropics (Caribbean)	light
<i>Psidium guajava</i>	common guava	Neotropics (Brazil)	tolerant ²
<i>Rhodomyrtus tomentosa</i>	downy rose myrtle	Asia	locally severe
<i>Syzygium cumini</i>	Java plum	Asia	light
<i>Syzygium jambos</i>	rose apple	Asia	severe
<i>Syzygium malaccense</i>	mountain apple	Polynesian introduction	minor
<i>Syzygium paniculatum</i>	brush cherry	Australia	locally severe
<i>Syzygium sandwicense</i>	'ōhi'a ha	Endemic to Hawai'i	tolerant ²

¹Primary sources: R. Anderson; R. Hauff; J. Uchida, pers. comm.

²Infection not observed in the wild or only single unconfirmed (identity) record, despite searches

just one more strain reaches Hawai'i, the consequences could be dire for 'ōhi'a. Each new genotype that would arrive has a substantial risk of increasing damage to 'ōhi'a, possibilities for mutation and/or genetic mixing, even with asexual strains, based on what is known about other *Puccinia* species (e.g., Steele and others 2001). Investigations are needed to clarify rust-*nioi* relationships. However, it is likely that keeping out new strains of *P. psidii* may be important for long-term survival of *nioi* as well as for the health of 'ōhi'a forest. The rust genotype present in Hawai'i has not yet demonstrated serious damage to *Eucalyptus* spp. or to many other Myrtaceae that have been affected by *P. psidii* elsewhere. In Brazil, both *Eucalyptus grandis* and *E. saligna*, the species most recommended by Whitesell and others (1992) for short-rotation plantations in Hawai'i, are known hosts of *P. psidii* (Simpson and others 2006). *E. grandis*, used extensively in plantations in Brazil by the paper industry, is one of the most susceptible *Eucalyptus* species to damage by *Puccinia psidii*; however, breeding of resistant *Eucalyptus* trees has been achieved (Junghans and others 2003).

The source of Hawaii's initial invasion by *P. psidii* is uncertain but is strongly suspected to have been decorative foliage of species in the myrtle family from the mainland USA, most likely California, where there had been outbreaks of this rust on cultivated myrtle (*Myrtus communis*) in 2005 (Mellano 2006). In 2006-07, Hawai'i Department of Agriculture (HDOA) inspectors on Maui intercepted several *P. psidii*-infected shipments of myrtle cut foliage, shipped from several California counties. The Department acknowledged the serious threat of the rust to Hawaii's one million acres of 'ōhi'a forests, and consequently to Hawaii's watersheds and biodiversity, based on a preliminary assessment of risk by Loope and La

Rosa (later published in 2008). Hawaii's Board of Agriculture unanimously approved an interim rule proposed by HDOA in August 2007 banning importation of plants in the myrtle family from "infested areas," specified as South America, Florida, and California. However, the interim rule has not been made permanent, and the Department has stated that it needs further information in order to formulate a long-term rule that imposes appropriate measures. A report by Loope (2010) is an attempt to meet that need.

Spores of this rust can survive for 2 to 3 months (Glen and others 2007); the pathogen can be transported to Hawai'i on Myrtaceae from anywhere in the USA, having originated in South or Central America, Caribbean Islands, Florida or California. There is much geographic reshuffling of flowers and foliage among the far-flung firms in the trade, especially for bouquet-making. Since guava rust is a non-reportable pest in the USA, foliage and flowers of the myrtle family can thus move freely, from state to state, throughout the country (Loope 2010).

Currently, the State of Hawai'i regulates incoming plant material in the family Myrtaceae by visual inspection. Inspection capacity and latent (asymptomatic) infections limit the ability to detect the rust. New molecular tests (e.g., the test developed by Langrell and others 2008 for Australia) could improve detection efficiency, but their cost and the time required to process samples currently precludes their routine use in ports of entry (such a test might be justified to assure that eucalyptus seeds allowed into the state by permit are not infected with rust). Interdiction has effectively kept coffee rust (*Hemileia vastatrix*) out of Hawai'i for 120 years and would offer the strongest protection for Hawai'i from *Puccinia psidii*.

PATHWAYS AND OTHER POTENTIAL PESTS OF HAWAII'S MYRTACEAE

International plantation forestry has much potential for facilitating invasions – especially insect pests and fungal pathogens infecting the widely used eucalypts (Ciesla and others 1996). Eucalypts are primarily Australian. The widely planted genera *Eucalyptus* and *Corymbia* comprise over 700 of the 4000+ species in the Myrtaceae. There are currently more than 16 million hectares of eucalypts planted in 80 countries around the world for a large number of purposes including pulp for paper manufacture, solid wood and structural timbers, and as woodlots for fuel (Lawson 2007). The largest areas planted in eucalypts are in India, Brazil and China. Movement of root pathogens among eucalypts has been much reduced by standard strict quarantine regulations that do not allow movement of soil, but pathogens of stems, leaves, and shoots have been introduced into new areas "with seeds and plant debris associated with seed" of forestry trees (Wingfield and others 2001). Little is known about the intercontinental spread of pathogens that infect solid wood products, but there is much potential for movement via this pathway (Wingfield and others 2001).

The cut foliage trade also provides a very significant but little-recognized pathway into Hawai'i from elsewhere, especially from California, the major exporter in the USA of Myrtaceae foliage, primarily ornamental juvenile leaves of *Eucalyptus* species. *Eucalyptus bridgesiana*, *E. cinerea*, *E. cordata*, *E. crenulata*, *E. gunnii*, *E. nicholii*, *E. parvula*, and *E. pulverulenta* are among the species used in the cut foliage industry in Australia (Barber and

others 2003). Insight is provided on cut foliage as a pathway for Australian eucalypt insects into New Zealand by Withers (2001): “[A] regulative loophole allowed the importation of untreated cut *Eucalyptus* foliage for the flower industry into New Zealand for some years up until 1999. Although all such foliage imports are inspected for the presence of unwanted organisms, inconspicuous life stages such as eggs and nymphs or young larvae of phytophagous insects may have passed the border controls undetected. It is not clear how long this particular loophole in New Zealand’s biosecurity net remained open.” More than 26 species of Australian eucalypt insects were established in New Zealand as of 2000 (Withers 2001). Lawson (2007) noted that from the early 1900’s introductions of eucalypt associated insects from Australia averaged about one every seven years, but this accelerated to one every 17 months between 1980 and 2000. New Zealand is an island country that is not a major grower of eucalypts, though eucalypts are a common urban amenity tree. Plantations of eucalypts form a small but important part of New Zealand’s timber industry.

California *Eucalyptus* stands were planted in the 19th century and benefited from over a century of essentially pest-free status. This situation ended in 1983-84 with arrival of the psyllid *Blastopsylla occidentalis* and the cerambycid borer *Phoracantha semipunctata*. Over the next quarter century, California’s Myrtaceae acquired numerous Australian insect species that feed on *Eucalyptus* spp. – including borers, defoliating beetles, psyllids, as well as insects that utilize the cultivated Australian brush cherry (*Syzygium paniculatum*), Geraldton waxflower (*Chamelaucium uncinatum*), and Brisbane box (*Lophostemon confertus*). California has about 90 species of non-native *Eucalyptus*, roughly the same number that occur in Hawai’i, and likely most of the same species. Biocontrol agents for many of them were introduced soon after their establishment. Perhaps surprisingly, Hawai’i and Florida have so far received a relatively low number of the Myrtaceae pests established in California (Table 2). This is fortunate for Hawai’i, which does not have the luxury of introducing biocontrol agents for pests of Myrtaceae so easily (see below).

Plant pathologist Michael Wingfield and a team of researchers at the Forestry and Agricultural Biotechnology Institute (www.fabinet.up.ac.za), University of Pretoria, South Africa, have made great strides in recent years in understanding the needs for pest management (especially of pathogenic fungi) for commercial forestry in Africa. They have developed and produced strong supporting evidence for a paradigm for how a pathway such as *Eucalyptus* forestry can potentially accumulate and disseminate pests, especially pathogens. The canker pathogen *Chrysosporthe austroafricana* is found on nonnative *Eucalyptus* (Myrtaceae) and *Tibouchina* (Melastomataceae), as well as on native *Syzygium cordatum* (Myrtaceae). This pathogen is hypothesized to be native to Africa (Nakabonge and others 2006). Research by Pavlic and others (2007) identified eight species of Botryosphaeriaceae as endophytes on native *Syzygium cordatum* in South Africa; pathogenicity trials conducted in greenhouses confirmed that all those species are potentially pathogenic to and pose a threat to local non-native *Eucalyptus* plantations. A native (South African) lepidopteran insect was discovered inflicting substantial damage to *Eucalyptus nitens* trees in South African plantations (Gebeyehu and others 2005). Thus, locally native pests that infect South African eucalypts can be inadvertently transported worldwide, as has been demonstrated for example by the recent establishment of the pathogen *Coniothyrium zuluense* in Hawai’i (Cortinas and others 2004).

Table 2. Non-native insects utilizing Myrtaceae that have invaded California (CA) over the past 26 years, with date of first record. All are native to Australia, though three were first described from California. Relatively few have already spread to Hawai'i (HI) or Florida (FL). Hosts are *Eucalyptus* spp. unless otherwise noted. Seven have been targets in California for biological control host testing and release.

Non-native insects utilizing Myrtaceae in CA	Date of first record
<i>Phoracantha recurva</i> (Newman)	CA 1995
Eucalyptus longhorned borer (Cerambycidae)	Biocontrol released for <i>P. semipunctata</i> in 1995 less effective for <i>P. recurva</i> ¹
<i>Phoracantha semipunctata</i> (Fabricius)	CA 1984 HI before 1972
Eucalyptus longhorned borer (Cerambycidae)	Biocontrol released in CA in 1995 ²
<i>Chrysophtharta m-fuscum</i> (Boheman)	CA 2003
Eucalyptus tortoise beetle (Chrysomelidae)	
<i>Trachymela sloanei</i> (Blackburn)	CA 1998
Eucalyptus tortoise beetle (Chrysomelidae)	Biocontrol released in CA in 2000 ¹
<i>Gonipterus scutellatus</i> (Gyllenhal)	CA 1994 HI 2004
Eucalyptus snout beetle (Curculionidae)	Biocontrol released in CA in 1994 ³
<i>Blastopsylla occidentalis</i> (Taylor)	CA 1983 HI 1999 FL2001
(Psyllidae)	
<i>Cardiaspina squamula</i> (Taylor)	CA ?
(Psyllidae)	
<i>Cryptoneossa triangula</i> (Taylor)	CA 1995
Lemon gum psyllid (Psyllidae)	
<i>Ctenarytaina eucalypti</i> (Maskell)	CA 1991
Blue gum lerp psyllid, blue gum psyllid (Psyllidae)	Biocontrol released in CA in 1994 ⁴
<i>Ctenarytaina longicauda</i> (Taylor)	CA ca. 1988
(Psyllidae)	Described as new species in 1987 in both Australia and CA ⁵ .
<i>Ctenarytaina spatulata</i> (Taylor)	CA 1991
(Psyllidae)	
<i>Glycaspis brimblecombei</i> (Moore)	CA 1998 HI 2001 FL2002
Red gum lerp psyllid (Spondyliaspidae)	Biocontrol released in CA in 1999 ⁶ Biocontrol agent first found in 2004 in HI ¹¹
<i>Trioza eugeniae</i> (Froggatt)	CA 1988
Eugenia psyllid (Psyllidae)	Biocontrol released in CA in in 1992 ⁷
<i>Eucalyptolyma maidenii</i> (Froggat)	CA 2000
Spotted gum lerp psyllid (Spondyliaspidae)	
<i>Epichrysocharis burwellii</i> (Schauff)	CA 1999 HI 2001
Lemon gum gall wasp (Eulophidae)	First described as new species in CA ⁸
<i>Oncastichus goughi</i> (Headrick and La Salle)	CA 1995
on waxflower, <i>Chamelaucium uncinatum</i>	First described as new species in CA ⁹
(Hymenoptera: Eulophidae)	
<i>Selitrichodes globulus</i> (La Salle & Gates)	CA 2008
(Eulophidae)	First described as new species in CA ¹⁰

¹Paine and Millar 2002

²Hanks and others 1995

³Hanks and others 2000

⁴Dahlsten and others 1998

⁵Taylor 1987

⁶Dahlsten *et al.* 2005

⁷Dahlsten and others 1995

⁸Schauff and Garrison 2000

⁹Headrick and others 1995

¹⁰LaSalle and others 2009

¹¹Daane *et al.* 2005

Documentation of movement of pests between non-native eucalypts and native Myrtaceae is also progressing in South America (for example, Pérez and others 2008). Lawson (2007) reported that more than 231 species of insects in 62 families had already been recorded on eucalypts in Brazil by 1981. Native vegetation in Brazil is rich in species of Myrtaceae, increasing the risk of host switching to *Eucalyptus*.

In contrast, no Myrtaceae are native to California (or to any states in the USA other than Hawai'i and Florida), and essentially no native California insects utilize *Eucalyptus* or other Myrtaceae species as a host (Paine 2008). The one exception is a cerambycid beetle, *Xylotrechus nauticus*, which is only rarely found colonizing dead eucalypt hosts (Paine 2008). It is important to recognize that biological control agents against the non-native pests of *Eucalyptus* in California have been found and released with apparent minimal need for testing against potentially vulnerable native insects. Safety of biocontrol introductions is increased if the chemistry of the exotic tree being protected is very different from that of the local native trees. An introduced parasite or predator often finds its hosts by responding to volatile oils specific to an exotic tree species, in this case *Eucalyptus* spp. (Van Driesche 2008). This response may not necessarily be the case for introduced pests of native Myrtaceae or of *Eucalyptus* and other non-native Myrtaceae in Hawai'i, where native Myrtaceae are a major component of the vegetation.

Internationally, there is a developing understanding of the potential for spread of pests from Australia, where most eucalypts are native. Gall-forming wasps in the Eulophidae comprise a remarkable Australian insect group, which are often cryptic within Australia but break out in enormous numbers when Australian Myrtaceae are transplanted to other locations in the world. For example, the genus *Ophelimus* Haliday contains 50+ described species, with many more that are undescribed; and it is almost exclusively restricted to *Eucalyptus* (Austin and others 2004). Eulophid gall wasps from Australian Myrtaceae have demonstrated an increasing tendency over the past quarter century to demonstrate invasiveness outside Australia before being “discovered” in Australia (Taylor 1987, Headrick and Redak 1995, Schauff and Garrison 2000, Mendel and others 2004, Protosov and others 2007, La Salle and others 2009).

Potential spread of diseases from Australia can be illustrated by the presence of more than 60 species in the ascomycete genus *Mycosphaerella* described from eucalypts from somewhere in the world; although, relatively few species are yet known to occur in native eucalypt forests (Carnegie and others 2007). This is probably in part because studies of fungi have been focused on eucalypt plantations, not native stands. Another likely factor causing species native to Australia to be found overseas first is that when introduced to susceptible, even-aged exotic plantations their impact is greater, disease symptoms obvious and they are described (Carnegie and others 2007).

In the long run, there is great potential for additional invasion of Hawai'i by many pests of *Eucalyptus* either directly from Australia or indirectly from areas where *Eucalyptus* forestry is practiced. Hawai'i can seek protection from USDA regulations, as well as through collaborative planning and State rule-making spurred by local stakeholders in Hawai'i.

Currently, we recommend to HDOA the option of interdiction of Myrtaceae from the continental USA, with the primary rationale of preventing additional strains of *Puccinia psidii* from establishing in Hawai'i, but with the important supplementary benefit of preventing establishment in Hawai'i of other very significant pests of multiple species of Myrtaceae that are already in the USA (or in the case of *Ophelimus maskelli*, is likely to be there very soon). These include *Trioza eugeniae*, the Eugenia psyllid (Hemiptera: Psyllidae), which attacks *Metrosideros excelsa* in California (Paine and Dreistadt 2007); *Chrysophtharta m-fuscum*, the Eucalyptus tortoise beetle (Coleoptera: Chrysomelidae) (Millar and others 2009); *Leptocybe invasa*, the blue gum chalcid wasp (Hymenoptera: Eulophidae) (Mendel and others 2004, Wiley and Skelly 2008); *Ophelimus maskelli*, a seriously damaging gall wasp (Hymenoptera: Eulophidae) (Protosov and others 2007); and the fungal pathogens *Mycosphaerella molleriana* (Ascomycota: Mycosphaerelliaceae, crinkle leaf disease of *Eucalyptus* spp.) (Kliejunas and others 2003) and *Neofusicoccum parvum* (Ascomycota: Botryosphaeriaceae), currently causing serious damage to *Syzygium paniculatum* in South Florida nurseries (Ploetz and others 2008). Each of these pests would be likely to cause very significant damage to native and/or cultivated Myrtaceae in Hawai'i. Each of these pests is a prime candidate for transport by the foliage and/or nursery stock pathways from California and/or Florida into Hawai'i.

CONCLUSIONS

The literature on the rust *Puccinia psidii* shows that there are numerous genetic strains with differential ability to infect suites of host plant species. A single genetic strain (apparently a single genotype) of the rust has been established in Hawai'i since 2005. The strain in Hawai'i causes more damage to the non-native tree rose apple, *Syzygium jambos*, than any strain yet seen, destroying its populations across the landscape, but only mildly affects 'ōhi'a, Hawai'i's dominant endemic tree. Every effort must be made to keep out new strains of *P. psidii* to protect 'ōhi'a forest. *P. psidii* is also considered to be the most serious threat to eucalypt plantations worldwide. The only way to prevent new strains of *P. psidii* seems to involve drastic restriction of Myrtaceae coming into the State – nursery stock, flowers and foliage, and genetic stock (seed or seedlings) of *Eucalyptus* for forestry purposes. Benefits of this policy would be substantial for reducing establishment of other pests of Myrtaceae, not just the rust. We recommend that biodiversity conservation and forestry interests work together with Hawai'i Department of Agriculture toward the necessary measures as soon as possible. We recommend that a diagnostic test for presence of *P. psidii*, already developed for use in Australia (Langrell and others 2008), be considered for use to assure that seeds of eucalyptus brought into Hawai'i are free of the rust.

Hawai'i Department of Agriculture has a clear mandate to protect Hawai'i's natural environment, forest industry, and cultivated Myrtaceae. Principles of the World Trade Organization's Treaty on Sanitary and Phytosanitary Measures and the International Plant Protection Convention are consistent with the right of Hawai'i to take action (Loope 2010). The current threat of *Puccinia psidii* and the other six serious threats to Myrtaceae is primarily via the continental USA; however, that may change in the future, as *P. psidii*

expands its range (e.g., Glen and others 2009). If Hawai'i was to decide on regulation to protect its native and introduced Myrtaceae, there is the prospect that USDA would assist by strengthening federal regulation of Myrtaceae from foreign countries.

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FOREST AND FORESTRY INSECT PESTS IN HAWAII: PAST, PRESENT, AND FUTURE

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ABSTRACT

Since the formation of the Bureau of Agriculture and Forestry in 1892, Hawai'i has experienced relatively few introductions of arthropod or pathogen pests of native forest or silviculture that have been persistently serious. Outbreaks of defoliating *Scotorythra* moths and koa wilt (*Fusarium oxysporum*) attack koa (*Acacia koa*) and can cause serious damage, but at least the former is presumed endemic. Since the arrival of Europeans, only a few very significant forest pests such as the Australian fern weevil (*Syagrius fulvitaris*), black twig borer (*Xylosandrus compactus*), koa psyllid (*Acizzia uncatoides*), and two-spotted leafhopper (*Sophonia rufofascia*) have arrived in Hawai'i, so that forests here have in general been spared attacks on valued dominant species of native forest plants. Since 2005, three new serious threats have appeared, erythrina gall wasp (*Quadrasticus erythrinae*), myoporum thrips (*Klambothrips myopori*) and a fungal pathogen called guava rust (*Puccinia psidii*), each attacking one or more valued dominant forest plant species in Hawai'i. These new pests are either a potential or present threat to valued dominant forest plants and could adversely affect entire plant communities or ecosystems on landscape levels. Several pests of our dominant forestry trees in the genus *Eucalyptus* have arrived over the years, with several showing up very recently. The ever increasing trans-Pacific movement of plant material can only increase over time the risks to dominant forest plant species. Biological Control has been used successfully on some of these pests but it was ineffective on others. It is the last hope of control for several of these including, some new ones. New forest protection biosecurity regulations are needed to prevent the attack and death of valued dominant forest plants in Hawai'i, as seen in recent decades in the Eastern USA.

INTRODUCTION

Hawaii's Central Pacific location as a transit point between sides of the Pacific Rim and among Pacific islands has brought it both commercial success as well as many of the plagues of international commerce and travel. The IUCN's Invasive Species Specialist Group created a list of "One Hundred of the World's Worst Invasive Alien Species"; Hawai'i has 50 of them. Almost as many non-native arthropods have become established in Hawai'i as in all of the other 49 states (McGregor 1973); likely reasons include Hawai'i's "Pacific hub" setting with high "propagule pressure," a benign climate, and a proximity of diverse habitats to ports of entry. Data from both Beardsley (1979) and the Hawai'i Department of Agriculture

(unpublished) indicated that the rate of establishment of new State records for insects was about 20 per year between 1961 and 1990. That rate is probably influenced by who is looking for new insects as much as by how much cargo and passenger traffic passes through the State. However, these are the best and most recent data we have so we must consider what it means. Many of the new introductions are innocuous species, but some are pests and some of these are significant forest or forestry pests in Hawai'i (Tables 1 and 2).

Table 1. Significant pests of native forest plants in Hawai'i.

Latin Name	Common Name	First Reported	Host Plants	Biocontrol Target?	Under some control by BC or adventives?
<i>Rhabdoscelus obscurus</i>	New guinea sugar cane weevil	1865	<i>Pritchardia spp.</i>	Y	?
<i>Syagrius fulvitaris</i>	Australian fern weevil	1903	<i>Cibotium spp.</i> , <i>Sadleria spp.</i>	Y	Y
<i>Xylosandrus compactus</i>	Black twig borer	1961	Polyphagous (44 fam.; 109 spp.)	N	N
<i>Acizzia uncatoides</i>	Koa psyllid	1966	<i>Acacia koa</i>	Y	Y
<i>Sophonia rufofascia</i>	Two-spotted leaf hopper	1982	Polyphagous (87 fam.; 307 spp.)	N	Y
<i>Specularius impressithorax</i>	Erythrina seed bruchid	2001	All <i>Erythrina spp.</i> in Hawaii	N	Y?
<i>Quadrastichus erythrinae</i>	Erythrina gall wasp	2005	All <i>Erythrina spp.</i> in Hawaii	Y	Under evaluation
<i>Klambothrips myopori</i>	Naio thrips	2009	<i>Myoporum sandwicense</i>	N	Under evaluation

Table 2. Significant pests of alien forest trees that have commercial forestry potential in Hawai'i.

Latin Name	Common Name	First Reported	Host Plants	Biocontrol Target?	Under some control by BC or adventives?
<i>Pineus pini</i>	Eurasian pine adelgid	1970	<i>Pinus spp.</i>	Y	?
<i>Phoracantha semipunctata</i>	Eucalyptus longhorned borer	1972	<i>Eucalyptus spp.</i>	N	?
<i>Ctenarytaina eucalypti</i>	Blue gum psyllid	1993	<i>Eucalyptus globulus</i> in Hawaii	N	Y?
<i>Glycaspis brimblecombei</i>	Red gum lerp psyllid	2001	<i>Eucalyptus spp.</i>	N	Y?
<i>Gonipterus scutellatus</i>	Eucalyptus snout beetle	2004	<i>Eucalyptus spp.</i>	N	Y?
<i>Ophelimus sp.</i>	Eucalyptus gall wasp	2005	<i>Eucalyptus spp.</i>	N	Y?

Here we define forest and forestry insect pests as any insect that appears to have caused potentially serious damage at a population level to either a native forest plant or any tree planted for commercial forestry use. We briefly discuss here each of the forest/forestry pests we consider significant, whether their damage to a valued forest plant was significant only in the past or still is. We intend this paper as a synopsis presenting basic information on specific

forest pests. We have divided Hawaii's forest pests into two groups: 1) those pests that appear to have damaged populations of important native forest species, and 2) those that have damaged populations of non-native forest species with potential economic importance. Here we review the past and recent insect pest threats to valued dominant native plants and to two genera of potentially economically important forest trees. We have only included one seed predator (*Specularius impressithorax*) in our discussion, but certainly there are others affecting valued dominant forest plants that could be discussed in a future paper.

Native forest insects in Hawai'i, in general, do not typically undergo large population outbreaks (Swezey 1954). A notable exception involves geometrid moths in the genus *Scotorythra*. Outbreaks of koa (*Acacia koa*) defoliation by the larvae of *S. paludicola* and apparently two other species of *Scotorythra* have been documented in the literature by Stein and Scowcroft (1985) and Haines and others (2009) and need not be discussed in detail here. This phenomenon is unusual for Hawai'i and is the only case in which native insects can be a serious forest pest of valued dominant forest tree species, though tree mortality has been rarely observed.

Given its well documented vulnerability to invasions, Hawai'i has been remarkably fortunate in that it has suffered from relatively few recognized non-native forest pests. For some forest pests, the year of introduction is uncertain, while most others are documented in the Proceedings of the Hawaiian Entomological Society or (since 1995) the Records of the Hawai'i Biological Survey (<http://hbs.bishopmuseum.org/pubs-online/bmop.html>). The first comprehensive publication that included documentation of insect pests of forest plants was "Forest Entomology in Hawaii" by O.H. Swezey (1954). It is still the most comprehensive and valuable reference for native forest insects, but with respect to forest pests it is now out of date. Overviews devoted to pests of koa (e.g., Stein 1983a, b, Gardner 1997) are more numerous than those for other forest species. Fullaway and Krauss (1945) have published on individual forest insects or other insect taxa, but there has not been a review of forest insects (nor forest pests in particular) since Swezey (1954). The aforementioned publications were not written to address forest insect pests exclusively, but more to document and discuss insects established in Hawai'i, both native and alien. It is our purpose here to review the forest pests in particular. We must also note here that Howarth (1985) has produced a comprehensive review of the impacts of alien land arthropods and molluscs on native plants, animals, and ecosystems in Hawai'i.

SERIOUS ARTHROPOD PESTS OF NATIVE FOREST SPECIES

***Rhabdoscelus obscurus* Boisduval (Dryophthoridae), new guinea sugarcane weevil**

Native to New Guinea and adjacent islands, *Rhabdoscelus obscurus* is found throughout the Pacific attacking sugar cane and palm species including coconut. The weevil lays its eggs in holes made by its proboscis in any part of the plant, and the hatched larvae bore into whatever plant tissue is present. Larvae burrowing within rachises cause leaves to break off weakening the plant. Those that reach the palm's terminal shoot can cause rapid death to the plant (Bianchi and Owen 1965). This weevil was reported causing damage to sugar cane in Hawai'i as early as 1865 (Pemberton 1964). In Hawai'i, the weevil also attacks palms in the

genus *Pritchardia*, the only native genus of palms which contains 19 species (Wagner and others 1990). Mortality of *Pritchardia* spp. caused by weevil infestation was observed in the Waimea Arboretum and Botanical Garden on O'ahu (David Orr pers. comm.), and the species has also been documented on naturally occurring *Pritchardia* on Huelo islet off the North Coast of Moloka'i (S. Montgomery pers. comm.). Once one of the most common trees throughout a wide range of ecosystems in Hawai'i, native species in the genus *Pritchardia* are now rarely found at low elevations except for some off-shore islands where rats (which consume palm fruit) are not present.

In the early 1900's, work on a biological control for *R. obscurus* resulted in the discovery of a tachinid parasite *Lixophaga sphenophori* (formerly *Microceromasia sphenophori*) in New Guinea. *Lixophaga sphenophori* was soon after brought to Hawai'i for rearing and release and became established in cane fields in Hawai'i reducing damage to sugar cane (Pemberton 1964).

***Syagrius fulvitaris* Pascoe (Curculionidae), Australian fern weevil**

This weevil has been in Hawai'i since at least 1900 (Perkins 1925). Pemberton (1964) reviews the early history of the weevil in Hawai'i. Its host range includes alien species of ferns in Hawai'i but also the native *Diplazium* spp., *Microlepia strigosa*, *Asplenium* spp., *Cibotium* spp. and the weevil is still especially damaging on *Sadleria* spp. Dark streaks caused by larval damage on the rachis are seen on most specimens in the forest. Rachises seem more brittle from attack. The constant feeding damage appears to stress the older ferns and may shorten their life span. Swezey (1912) reported *Sadleria* ferns apparently killed by the weevil in Makiki Valley, O'ahu. Pemberton (1964) noted that an unsuccessful eradication attempt for the weevil had been made on the island of Hawai'i, at 'Ōla'a.

A single braconid wasp parasitoid species, *Doryctes syagrii* (Fullaway), was released for biological control in 1921 and became established (Pemberton 1921). Pemberton (1964) later briefly mentioned that the parasitoid was exerting control, but that more natural enemies are needed. That latter comment applies to this day. Fullaway (1921) reports also that the braconid wasp *Ontsira (Ischiogonus) palliatus* (Cameron) has been reared out from the weevil and that a fungus also attacks the weevil in the wild.

***Xylosandrus compactus* (Eichhoff) (Scolytidae), black twig borer**

The black twig borer (BTB), *Xylosandrus compactus* is native to Asia, and became established in the Hawaiian Islands in or before 1961 (Nelson and Davis 1972). The species was first discovered on O'ahu, and despite inter-island import restrictions, BTB was established on Hawai'i Island by 1963 (Davis 1963).

The species is polyphagous, with a host range that includes a diversity of economically important plant species used in agriculture, horticulture, and forestry (Nelson and Davis 1972, Hara and Beardsley 1979). Most notably, the species is a significant economic pest of coffee, and has spread into many regions of the world where coffee is grown (Davis 1963, Nelson and Davis 1972, Hara and Beardsley 1979). In Kona, BTB has become a common pest, and during heavy infestations the species has been documented to infest up to 20% of all coffee branches (USDA 2009).

In Hawai'i, BTB has also become a pest of the native koa tree (*Acacia koa*: Fabaceae). *Koa* is a valued dominant tree species which constitutes a major component of native Hawaiian forests (Baker and others 2009). The impacts of BTB infestations on koa are currently unknown; however, the cumulative damage caused by black twig borer in conjunction with other koa pests is documented to result in tree mortality (Daehler and Dudley 2002, Baker and others 2009). Specifically, the BTB is frequently found in koa which are infected by koa wilt, an infection believed to be caused by a soil-borne fungus (*Fusarium oxysporum*) (Daehler and Dudley 2002).

Given the wide host-range, BTB is likely adversely impacting additional native Hawaiian plant species. The pest has been observed to infest *Sapindus saponaria* (Sapindaceae), *Pittosporum sp.* (Pittosporaceae), *Metrosideros polymorpha* (Myrtaceae), *Canavalia napaliensis*, *Caesalpinia kavaense* and *Acacia koaia* (Fabaceae), *Gardenia mannii* and *G. brighamii* (Rubiaceae), *Melicope Hawaiensis* (Rutaceae), *Pteralyxia kauaiensis* (Apocynaceae), and *Claoxylon sandwicense* (Euphorbiaceae) in addition to threatened and endangered plant species including *Colubrina oppositifolia* (Rhamnaceae), *Alectryon macrococcus* (Sapindaceae), *Flueggea neowawraea* (Euphorbiaceae), *Caesalpinia kavaensis* (Fabaceae), *Sesbania tomentosa* (Fabaceae), *Melicope mucronulata* and *M. knudsenii* (Rutaceae) (Wagner and others 1985, HRPRG 2009). It is possible that the BTB is at least partly responsible for driving several of these species toward extinction.

The BTB has the unusual ability to infest healthy trees, as well as trees that are biologically stressed. The beetles bore into the center of healthy twigs, and inoculate the plant with spores of an ambrosia fungus (*Fusarium solani*) before laying their eggs (Davis 1963, Nelson and Davis 1972). Upon emergence, the BTB larvae begin to feed on the growing ambrosia fungus. Twig and branch dieback results as a consequence of tunnel excavation and the introduction of the ambrosia fungus [as well as other pathogens], as opposed to directly from feeding damage (Davis 1963, Nelson and Davis 1972). A BTB infestation is characterized by the presence of small entry/exit holes, twig browning and leaf-drop, and twig/branch mortality. Mortality of an entire plant can result from heavy infestations (Davis 1963, Nelson and Davis 1972).

Black twig borer control methods are currently being developed and tested by researchers at the University of Hawai'i (USDA 2009). The research is directed at quantifying the efficacy of various beetle trap types and lure types (e.g. eugenol, alpha pinene, ethanol), and has already informed black twig borer management efforts in Hawai'i.

***Acizzia uncatoides* (Ferris & Klyver) (Psyllidae), acacia psyllid**

The acacia psyllid was first detected in Honolulu in a mosquito light trap in 1966 (Joyce 1967). Believed to be native to Australia it had been originally described in New Zealand where acacias are exotic. It was detected in California in 1954 damaging ornamental *Acacia* and *Albizia spp.* (Koehler and others 1966).

In Hawai'i the acacia psyllid attacks the native koa tree (*Acacia koa*). Koa is one of Hawaii's most important tree species. It has important cultural values for native Hawaiians, for

example being the preferred species for canoes. It also plays an important ecological role as one of Hawaii's valued dominant forest trees and a provider of habitat for many of Hawaii's remaining native forest birds and other fauna. It is also a highly desirable wood for timber and woodworking, although current supplies are limited.

The psyllid feeds and breeds on new terminal growth causing dieback. Outbreaks correspond to new growth flushes on trees (Leeper and Beardsley 1976). Large populations of the psyllid were noted in an elevational survey of canopy arthropods on Mauna Loa on the island of Hawai'i carried out in the 1970's (Gagné 1979).

Although predators were observed attacking *A. uncatoides*, none appeared to provide effective control (Leeper and Beardsley 1976). Two coccinellids were introduced at sites where *A. uncatoides* was infesting koa (Leeper and Beardsley 1976). One of them, *Harmonia conformis*, exerted effective control at one of the sites but failed to establish at other sites. The other *Diomus pumilio* never established at the research sites.

While *A. uncatoides* infestations still occur sporadically it appears to be under control in most places. However, by attacking and sometimes killing new terminal growth, the acacia psyllid may contribute to bushy growth forms typically found in planted koa at higher elevations (Baker and others 2009), thereby reducing the commercial value of the trees. In addition *A. uncatoides* is implicated for spreading native koa rusts (*Uromyces spp.*) which can also damage tree growth form by producing witches' brooms (Leeper and Beardsley 1973).

***Sophonia rufofascia* Kuoh and Kuoh (Cicadellidae), the two-spotted leafhopper**

Originally described from southern China, two-spotted leafhopper (TSL) was discovered on O'ahu in 1987 after being detected on plants exported to California (Heu and Kumashiro 1989). By 1995, the leafhopper was widespread on the six largest Hawaiian Islands and distributed from sea level to an elevation of 1675 m (Fukada 1996, D. Foote unpublished data). TSL was observed to attack over 300 plant species in 83 families, of which 68% were economically important fruit, vegetable, and ornamental crops, and 22% were endemic to the Hawaiian Islands (including 14 rare and endangered species). Leafhopper feeding and oviposition cause plant vascular bundle abnormalities, resulting in interveinal chlorosis, vein browning, retarded development of new growth, and even death of affected plants; damage to vascular bundles is caused by oviposition into midveins (Jones and others 2000).

In the 1990's, TSL outbreaks caused dieback of uluhe (*Dicranopteris linearis*), a climbing fern that covers large areas of watershed on O'ahu, raising public concern. Additionally hapu'u tree ferns (*Cibotium spp.*) on O'ahu suffered greatly from TSL. There are now many fewer of these plants, and those that remain are often diminished in vigor and frond size (Palmer 2003). 'Ōhi'a (*Metrosideros polymorpha*), the valued dominant tree in most of the remaining native Hawaiian forests is also a host, and TSL has been associated with dying 'ōhi'a trees, especially at lower elevations (Jones and others 2000).

TSL has also significantly attacked two other valued dominant native Hawaiian plant species -- a'ali'i (*Dodonaea viscosa*) and ohelo (*Vaccinium reticulatum*) (Johnson and others 2001,

Lenz and Taylor 2001). Leafhopper invasion of native ecosystems at Hawai'i Volcanoes National Park is facilitated by the invasive tree *Morella faya*, a species associated with a nitrogen-fixing actinorhizal symbiont and consequently a very high foliar nitrogen content. The buildup in leafhopper numbers on *M. faya* has resulted in their dispersal to other plant species in the area; Lenz and Taylor (2001) reported 2 to 19-fold higher TSL populations on 'ōhi'a and a'ali'i within sites invaded by *M. faya* compared to the sites where this weed was removed.

In field surveys conducted on the island of Hawai'i, Yang and others (2002) and Johnson and others (2001) discovered that eggs of TSL are attacked by two species of adventive parasitoids, *Chaetomyrmar* sp. and *Schizophragma bicolor* (Hymenoptera: Mymaridae), and by several species of endemic Hawaiian parasitoids in the genus *Polynema* (Hymenoptera: Mymaridae). A *Chaetomyrmar* sp. was found to be by far the most important one that parasitizes *Sophonia* in southern China (Messing and others 2003); Johnson and others (2001) speculated that since it was first collected in Hawai'i in 1995, it may have originally arrived from China with TSL; *Schizophragma* sp. had been collected as early as 1963 in Honolulu and was probably introduced from North America. Observed parasitism in Hawai'i was initially much lower than in China but Yang and others (2002) found that on certain hosts, in some habitats, parasitism in Hawai'i can be as high as 80%. TSL is assumed to be largely under effective control by parasitoids as outbreaks are rarely reported by forest managers.

Dieback of *Dicranopteris linearis* attributed to feeding by TSL took place especially on wet, open valley slopes and ridgelines of Maui, O'ahu, and Kaua'i. TSL damage to *D. linearis* was much less apparent on Hawai'i island; the difference was correlated with pubescence on leaves of the fern on the Big Island vs. glabrous leaves on the other islands (Jones and others 2000). Follett and others (2003) found that dead patches of *D. linearis* were colonized by both native and alien plant species. Recolonization of dead patches by live *D. linearis* spreading from the margins was common. *Clidemia hirta* and *Nephrolepis multiflora* were the most common invasive species colonizing and spreading in dieback patches.

***Specularius impressithorax* (Pic) (Chrysomelidae: Bruchinae), erythrina seed bruchid**
Hawaii's dry forest ecosystems have been drastically reduced by development, ungulate browsing, non-native grass invasions, and fire. Nevertheless, the endemic wiliwili tree, *Erythrina sandwicensis*, persists in large numbers as the valued dominant species of many low-elevation Hawaiian dryland forests. The species is a prolific seed producer, and seed predation was virtually absent until 2001. The African bruchine chrysomelid, *Specularius impressithorax* was first detected in Hawai'i in 2001 and became established on all main islands within the next two years (Samuelson and Medeiros 2006). The mode of entry for this invasive seed predator into Hawai'i is uncertain, but likely occurred with importation of trinket jewelry from Africa containing brightly-colored *Erythrina* seeds. Establishment of the beetle likely occurred on a non-native host, the widely cultivated *Erythrina variegata*. Within three years, *S. impressithorax* accounted for 77% mean seed crop loss in 12 populations of wiliwili on six main Hawaiian islands. (Medeiros and others 2008). However, by the time of the establishment of the *Erythrina* gall wasp in Hawai'i in 2005, seed crop loss of *E.*

sandwicensis had declined to < 10%, apparently due to parasitism of *S. impressithorax* by non-native parasitoids (A.C. Medeiros, pers. comm.).

***Quadrastichus erythrinae* Kim (Eulophidae), erythrina gall wasp**

The Erythrina Gall Wasp (EGW), *Quadrastichus erythrinae*, was discovered on the island of O‘ahu in April 2005. The purported native range of the EGW is east Africa. It was apparently introduced to Mauritius and Reunion Islands (Kim and others 2004) where it was first identified as a pest, and has spread at an incredible rate across Asia and the Pan-Pacific Islands (Heu and others 2005). Once introduced to Hawai‘i, the EGW spread across all of the main Hawaiian Islands within a period of only six months.

EGW infestation has resulted in chronic defoliation and mortality of thousands of *Erythrina* trees, including *E. variegata* and the endemic wiliwili tree, *E. sandwicensis*. Since becoming established the EGW is generally observed in high densities on *Erythrina* trees, and especially so when the trees have recently flushed with new foliage (Heu and others 2005).

Damage to *Erythrina* occurs as a direct result of the EGW life cycle. Female EGW’s oviposit eggs into new growth, specifically favoring new leaves and stems (Heu and others 2005). When wasp larvae emerge from eggs and begin feeding on the plant tissue, they trigger the development of galls, malformed areas of thickened tissue, around wasp larval feeding sites. Gall formation results in the thickening and curling of leaves, and the swelling of petioles and stems. It is common for leaf drop and branch dieback to occur during, and following, heavy EGW infestations (Heu and others 2005). All of these symptoms directly interfere with *Erythrina* growth, and have resulted in significant mortality in both native and introduced *Erythrina* species.

While research on chemical controls for EGW commenced immediately and with some success (with imidacloprid in particular) (Xu and others 2006, Doccola and others 2009), resource managers in Hawai‘i soon realized that such controls were impractical for wide-scale application. Consequently, researchers from the Hawai‘i Department of Agriculture (HDOA) and the University of Hawai‘i imported natural enemies of EGW from East Africa to explore the feasibility of controlling the species using classical biological control. Two wasps, *Eurytoma erythrinae* Gates & Delvare, (Hymenoptera: Eurytomidae) (Gates and Delvare 2008) and *Aprostocetus exertus* La Salle (Hymenoptera: Eulophidae) (La Salle and others 2009), were selected to undergo the extensive risk assessment process. HDOA concluded host-range testing for *E. erythrinae* in January 2007, and after obtaining approval from USDA and the State of Hawai‘i, field releases were initiated in November 2008.

HDOA unpublished monitoring data show that *E. erythrinae* has become established on each of the main Hawaiian islands. The parasitoid appears to be exerting high rates of parasitism on the pest, and *E. sandwicensis* trees are recovering in most parts of the State (HDOA unpublished data). The laboratory assessment by HDOA of the second natural enemy continues and is near completion.

***Klambothrips myopori* Mound & Morris (Phlaeothripidae), naio thrips (*myoporum* thrips)**

The thrips *Klambothrips myopori* Mound & Morris, was first collected in Orange County, California, in 2005 attacking *Myoporum laetum*; a species native to New Zealand that has been widely used for landscaping and roadside plantings since the 1950's (Downer 2006, Mound and Morris 2007). The California Invasive Plant Council (2006) has considered *M. laetum* to be invasive in coastal, riparian and woodland areas. The thrips was described by Mound and Morris (2007) as a new species, but is postulated to be native to New Zealand or Australia. It also is reported to attack *Myoporum* "pacificum" a creeping ornamental hybrid in California. Adults and larvae feed on younger leaves and cause pronounced deformation referred to by some as galls. It has killed large numbers of *M. laetum* in California already.

In Hawai'i, *K. myopori* was first collected on the island of Hawai'i in Waikoloa Village in South Kohala in March 2009 (Conant and others 2009). Since that time, it has been collected from Hapuna, Maunalani Resort (Kalahuiupuaa) and Puu Kapu (near Waimea) in South Kohala, and at Kona Village Resort (Kaupulehu) and Kona Palisades subdivision in North Kona. At the former three sites, it was collected on the prostrate form of Hawaiian naio (*Myoporum sandwicense*), "naio papa", which is now used extensively in resort landscaping. More recently, as of March 2010, it has been found in Waimea town, Pelekane Gulch in South Kohala and even up to Pohakuloa on the Saddle between Mauna loa and Mauna kea mountains at 4,500 ft., all on the tree form of naio (HDOA unpublished records).

The thrips has probably been spread by landscape maintenance crews moving from one job site to another within and between resorts. At the latter two sites, the thrips was collected from the tree form of naio. It was so far only known from the North Kona and South Kohala districts but in April 2010 it was found in a small landscape planting of naio papa in downtown Hilo (HDOA unpublished records). It has not been found in surveys of the other main islands. Since this thrips is known from as far North as 38°N latitude in California (Kitz 2006), it can apparently tolerate cooler winter temperatures, suggesting it could attack naio at high elevations (up to 10,000 ft.) in Hawai'i.

HDOA is in the process of surveying infested sites for predators such as anthocorid bugs. The Cuban laurel thrips on banyan (*Ficus microcarpa*) was apparently brought under biological control (Funasaki 1966) after release of the anthocorid *Montandoniola morguesi* Puton, so it is possible that this predator or others could bring the pest under some level of control. It is unfortunate that a native plant that had been widely used by the landscaping industry may now be too expensive to maintain in landscaping by necessary insecticide applications.

Aside from its status as a pest of landscaping plants, *K. myopori* is a potential great threat to the tree form of naio and the forests in which naio is a dominant component, such as the mamane-naio dry forests on Mauna kea. Naio is co-dominant with mamane (*Sophora chrysophylla*) on the western and southern slopes of that mountain. Two endangered birds, the akiapolaau (*Hemignathus munroi*) (Scott and others 1986) and palila (*Loxioides bailleui*) occupy these forests. The palila is restricted to these forests, since it is an obligate feeder on the seed pods of the mamane tree, and it also feeds on naio fruit (Banko and others 2002). The importance of naio to the palila is not known, but surely the forest would be altered if

naio was greatly reduced in abundance from thrips attack. On going browsing by wild sheep (*Ovis aries*) and mouflon sheep (*Ovis musimon*) has greatly reduced the density of mamane in relation to naio (Hess and others 1999), so that if naio density is reduced by thrips damage, the forest may be permanently altered. *Myoporum sandwicense* var. *degneri* is restricted to the leeward slope of Haleakala, between Ulupalakua and Kaupo Gap, and is already adversely affected by long term feral goat damage to the habitat and could be threatened even more seriously by the thrips.

SERIOUS ARTHROPOD PESTS OF SOME NON-NATIVE FOREST SPECIES WITH POTENTIAL ECONOMIC IMPORTANCE

***Pineus pini* (Macquart) (Adelgidae), eurasian pine adelgid**

This pest of conifers was first found on the island of Hawai'i in 1970 on *Pinus pinaster* (Funasaki 1971). Culliney and others (1988) cite published accounts that it was on all the main islands (except Lana'i) by 1978. They describe its native range as northern temperate ranges of the Holarctic and Oriental regions. It has been accidentally introduced into at least the United States, Australia (Simpson and Ades 1990) and Africa (Odera 1991). Host plants in both its native and adventive range are all within the genus *Pinus* (Havill and Footit 2007). The adelgid attacks the terminals, causing chlorosis and shoot death, and has killed healthy trees in Africa (Odera 1991). In Hawai'i, damage to pines was initially severe so the Hawai'i Department of Agriculture attempted eradication by chemical/mechanical means, but failed. A biological control program was begun and the Chamaemyiid fly *Leucopis obscura* Haliday was imported into quarantine from France and released in 1976. A second species in that genus, *L. nigriluna* McAlpine, was imported from Pakistan and released in 1972 and became established. The work by Culliney and others (1988) indicated that populations of *L. obscura* were positively correlated with those of the prey *P. pini* and they noted improved health of pines on Maui subsequent to release of the natural enemy (Nakao and others 1981). Greathead (1995) reports that specimens previously identified as *L. obscura* collected on Maui have since been identified as being *L. tapiae* Blanchard. However, it is possible that *L. obscura* was released and is established in the State.

Insect Pests of Eucalyptus in Hawai'i

Very extensive plantings of non-native *Eucalyptus* species were made in Hawai'i, primarily on degraded lands of the five main Hawaiian Islands, with most planting activity in the 1930s and 1960s. The proper role of *Eucalyptus* for Hawai'i is still uncertain, but options should arguably be kept open. *Eucalyptus* (ca. 90 spp. have been introduced to Hawai'i) has been out of favor, some would say neglected, as a resource for the past quarter century. *Eucalyptus* spp. in Hawai'i, having not yet accumulated a serious array of forest pests, may provide a potentially very important natural resource in the future –for production of lumber, specialty woods, biofuels, and perhaps decorative foliage (Loope and La Rosa, this volume). The most serious eucalypt pests found in Hawai'i to date are discussed below.

***Phoracantha semipunctata* F. (Cerambycidae) eucalyptus longhorned borer.** *Phoracantha semipunctata*, one of the eucalyptus longhorned borers native to Australia, was the first

eucalypt pest to establish in the Hawaiian Islands; it was present on O'ahu and Kaua'i by 1972 (Gressitt and Davis 1972) and had spread to Maui by 1992 (Whitesell and others 1992). Whitesell and others (1992) stated that it was not considered a serious threat to the eucalyptus plantations by local entomologists. Occasional tree death has been observed on Maui during extreme drought conditions (Robert Hobdy, pers. comm.). But *Eucalyptus globulus*, generally known as particularly susceptible to *P. semipunctata* elsewhere, seems to escape significant impacts on Maui despite being invasive there.

In 1984, *P. semipunctata* became one of the first eucalyptus pests found in California, where it raised much concern. Within a few years it was causing severe kills of various eucalyptus species from San Diego County north to the San Francisco Bay area. Similar impacts by *P. semipunctata* had occurred in most regions in the world where eucalypts had been planted including Portugal, Spain, Italy, Israel, Egypt, Tunisia and South Africa (Hanks and others 1996). The response in California was to find and eventually introduce *Avetianella longoi*, a highly specific egg parasitoid of *P. semipunctata* and several closely related Australian beetles, in native habitat in Australia. The parasitoid spread rapidly, brought *P. semipunctata* under control, and was subsequently introduced in other parts of the world where *P. semipunctata* had been damaging eucalypts (Hanks and others 1996).

Given the notorious impact of *P. semipunctata* in many, if not most, locations outside Australia, and the parasitoid is not established in Hawai'i, why has the impact of *P. semipunctata* in Hawai'i gone almost unnoticed to date? Paine (2008) tentatively attributes the modest impact of the beetle in such locations as Hawai'i and Brazil to significantly higher moisture content of the bark of eucalypts in locations without the extreme summer drought characteristic of classic Mediterranean climates. Hanks and others (1999) found that supplemental summer irrigation in California prevented host susceptibility.

Currently there is no evidence that the egg parasitoid of *Phoracantha semipunctata* has reached Hawai'i. However, biocontrol organisms, probably from California, have possibly arrived by chance for the three potentially major pests of Eucalyptus discussed below.

***Ctenarytaina eucalypti* (Maskell), blue gum psyllid (*Psyllidae*).** *Ctenarytaina eucalypti* was first reported in the USA in California in Monterey County in January 1991; by summer it had spread to Southern California. This psyllid infests Eucalyptus species that have waxy blue juvenile foliage, such as blue gum (*Eucalyptus globulus*). Damage in California was most significant on foliage of silver-leaved mountain gum, also called baby blue gum (*Eucalyptus pulverulenta*), grown commercially for floral arrangements. "Within months, psyllid populations in the coastal regions reached extremely high levels, resulting in blemished baby blue gum foliage that often could not meet the market's high cosmetic standards" (Dahlsten and others 1998). Growers responded with increasingly frequent insecticide applications in 1991 and 1992. Insecticide treatments proved to be expensive and unreliable.

About 30 ornamental eucalyptus growers funded a "Eucalyptus Growers Committee" to investigate new strategies to combat the psyllid, including a classical biological control program. "At their request, University of California Berkeley entomologist Donald Dahlsten

conducted a search for natural enemies of the psyllid in Australia and New Zealand in late 1991 and early 1992, and shipped back a parasitoid, *Psyllaephagus pilosus* Noyes (Encyrtidae), which attacks only *C. eucalypti*. After its release in California in late 1992, this parasitoid spread rapidly throughout the ornamental eucalyptus-growing region. Reductions in psyllid populations were immediate and dramatic, and insecticide treatments for this pest virtually ceased by 1995 (Dahlsten and others 1998).

Ctenarytaina eucalypti has also invaded New Zealand, Papua New Guinea, Sri Lanka, Madeira, Canary Is., Europe (Portugal and Spain, Germany, United Kingdom, Ireland), Africa (South Africa, Burundi, Tanzania, and Ethiopia) and South America (Bolivia, Brazil, Colombia). In Brazil, the species most affected are *E. globulus*, *E. maidenii*, *E. bicostata*, *E. dunnii* and *E. nitens* (Santana and Burckhardt, 2007). Severity of damage apparently differs in different locations but it has been serious enough that the biocontrol parasitoid has been introduced in many locations (e.g., Chauzat and others 2002). According to Ciesla and others (1996), *C. eucalypti* was considered in the mid-1990s the most important (damaging) forest insect in Portugal.

The blue gum psyllid was first noted in Hawai'i in HDOA surveys in 1993. It is likely to have arrived in Hawai'i from California via *Eucalyptus pulverulenta* in the foliage trade. In the quarter century since its arrival, it has attracted almost no attention, perhaps because the biocontrol parasitoid may have arrived with the original introduction of the psyllid from California. However, the parasitoid *Psyllaephagus pilosus* was not actually collected in Hawai'i on blue gum psyllid until December 2002, at Olinda, Maui (M. Fukada pers. comm.)

***Glycaspis brimblecombei* (Spondyliaspidae), red gum lerp psyllid.** *Glycaspis brimblecombei* established in southern California by mid-1998; by 2000, it had spread throughout the state and was causing severe damage (with millions of dollars in damage and control costs) to *Eucalyptus camaldulensis*, river red gum, one of the most commonly planted shade and windbreak trees in California (Dahlsten and others 2005). Nymphs of *G. brimblecombei* build white conical shelters called lerps from excreted honeydew and waxes, and live underneath these structures. The nymphs feed by sucking plant sap from leaves. Heavy infestations in California resulted in defoliation, branch dieback and occasional tree death (Paine and others 2000). A biological control program was initiated promptly, and a *G. brimblecombei*-specific (at least in California, vs. other psyllids) parasitic wasp was isolated from material ("mummified" lerps of *G. brimblecombei*) imported from near Adelaide, Australia, in 1999 and released beginning in September 2000. Through January 2003, 48,582 adults of *Psyllaephagus bliteus* were released in 78 release sites located in 42 counties (of the total of 58 California counties) throughout the range of *E. camaldulensis* in California (Dahlsten and others 2005). Psyllid densities decreased by 79% within slightly over a year in southern California, but less so farther north. Reduction in damage to trees was very high in coastal areas, but lower inland where winter temperatures are colder. Details are given in Dahlsten and others (2005).

Glycaspis brimblecombei was first detected in Hawai'i at Ulupalakua, Maui, in March 2001 by a Hawai'i Department of Agriculture entomologist (Nagamine and Heu 2001). It was collected again at Waimanalo, Oah'u, in July 2001. Nagamine and Heu (2001) stated:

“Presumably, the psyllid will have some effect on *Eucalyptus* in Hawai'i, particularly the preferred river red gum eucalyptus. According to Little and Skolmen (1989), river red gum eucalyptus is one of the most commonly planted eucalypts in Hawai'i and is frequently planted for windbreaks.” Daane and others (2005) noted that verified *P. bliteus* specimens have been reared from *G. brimblecombei* on a *Eucalyptus* sp. from Ulupalakua, Maui, Hawai'i, in 2004. It appears that impact of *G. brimblecombei* in Hawai'i has likely been minimal because the parasitoid was either introduced to Hawai'i initially with its host from California or arrived soon afterward.

***Gonipterus scutellatus* Gyllenhal (Curculionidae), eucalyptus snout beetle.** The Eucalyptus snout beetle, *Gonipterus scutellatus*, an Australian weevil was first noted in Hawai'i by Haines and Samuelson (2006) from *Eucalyptus robusta* in Kokomo (elevation 487m), Maui, in March through May, 2004. Larvae and adults were found within a fairly small area; Haines searched nearby stands of *Eucalyptus* in the vicinity of Kokomo, Makawao, and Olinda, Maui, for larvae and adults, but found no other populations. He considered it “likely that the weevil is present at other locations on Maui, since it was abundant at the site of collection, and since adults of this species are strong fliers living 2–3 months (Hanks and others 2000).” In response to a query in September 2009, Haines responded that he had noted it as highly abundant in the Hosmer Grove parking lot at ca. 2000m elevation in Haleakala National Park, Maui, in June 2009, but had not otherwise seen *G. scutellatus* beyond his original observations in Kokomo (W.P. Haines, email to L. Loope, 9-6-09).

Gonipterus scutellatus had already been known as a notorious defoliator of eucalypts in New Zealand, Africa, the Mediterranean area, and South America, when it was first discovered in Ventura County, California, in March 1994. Adults and larvae were found in California to prefer the foliage of *Eucalyptus globulus* and *E. viminalis*; the consumption of young and tender leaves, buds, and shoots leading to contorted growth, and eventually branch mortality. A selective and effective biological control agent for *G. scutellatus*, the egg parasitoid *Anaphes nitens* Giraud (Mymaridae), had effectively controlled this pest in many parts of the world. The effort to introduce this agent to California was relatively straightforward. Several hundred parasitized egg capsules of *G. scutellatus* from South Africa were received in late July 1994, which in a quarantine containment facility “yielded 100 adult *A. nitens* but no other primary or secondary parasitoid species” (Hanks and others 2000). The first small release of pure *A. nitens* occurred in August 1994, at the site of the original infestation in Ventura Co., less than 6 months after the original detection. *A. nitens* was well established in several southern California counties by mid-1997 and was spreading in tandem with its host. The wasp proved to be effective in suppressing California *G. scutellatus* populations, killing 95% of the eggs (Hanks and others 2000). *Anaphes nitens* has been effective in reducing damage by *G. scutellatus* in all parts of the world where it has invaded and attracted attention, with the exception of cooler highland areas in South Africa and in Spain (Loch 2008).

There is apparently no record to date of the parasitoid *A. nitens* in Hawai'i, but lack of obvious and extensive damage to *Eucalyptus* spp. on Maui suggests that the parasitoid may already have arrived on its own. Effort to confirm or deny this possibility is a priority for the near future.

***Ophelimus* sp. (Eulophidae), a *Eucalyptus* gall wasp.** A species of eucalyptus gall wasp in the genus *Ophelimus* was discovered by Mach Fukada of HDOA (pers. comm.) on the Island of Maui in August 2005. The species has yet to be identified, but John La Salle of CSIRO Entomology determined that it is likely congeneric with two well documented eucalyptus gall wasp pest species: *Ophelimus maskelli* (Ashmead) and *Ophelimus eucalypti* (Gahan).

Populations of eucalyptus gall wasps increase to high densities in areas where they are free from pressure by natural enemies. Damage to eucalyptus can be severe, with trees experiencing loss of crown structure through foliage desiccation and leaf drop (Protasov and others 2007b). These symptoms result directly from eucalyptus gall wasp larval feeding. Female wasps oviposit eggs into leaf tissues, which induce the development of blister-type galls on the leaf surface (Arzone and Alma 2000, Protasov and others 2007a). Within three years of the arrival of *O. maskelli* in Israel, galls were documented to cover two-thirds of the leaf volume in research sampling plots (Protasov and others 2007a).

There has been no formal monitoring of *Ophelimus* damage to *Eucalyptus* in Hawai'i; therefore, levels of infestation and current distribution of the pest remain undocumented. To date, impacts on *Eucalyptus* appear to be moderate in comparison to infestations in Europe and New Zealand by the two related eucalyptus gall wasp species. However populations of *Ophelimus* in Hawai'i are believed to be under some natural control by another introduced wasp. When initial *Ophelimus* collections occurred in 2005, a parasitoid wasp was also reared from blister galls, and tentatively identified by J. La Salle to be in the genus *Neochrysocharis* or *Closterocerus* (Eulophidae). The simultaneous introduction of a natural enemy may have prevented *Ophelimus* sp. from becoming a significant pest of *Eucalyptus* in Hawai'i.

DISCUSSION

Hawai'i is well known as the extinction capital of the United States, possessing one-fourth of the species federally listed as endangered. What is not generally appreciated is that much of Hawaii's unique biological heritage remains and can be protected with careful management. Large tracts of near-pristine ecosystems remain at high elevations. Even with the high incidence of extinction and endangerment in the Hawaiian Islands, Hawai'i has more non-endangered endemic species of vascular plants, birds, and insects than any other state except California (Loope 1998).

Biological control will undoubtedly be the the most practical method used against well established significant arthropod forest pests in the future as it has been in the past. It has successfully controlled some of these pests present in Hawai'i. It can and will have some element of risk to native relatives of target pests. In the case of eucalyptus pests, biological control of Myrtaceae pests is more complicated in Hawai'i than in California partly because of concerns about native psyllids on the native *Metrosideros polymorpha*, also in the Myrtaceae. This concern has been touched upon by Van Driesche (2008). Biocontrol has been increasingly scrutinized in Hawai'i since 1980 (Howarth 1983), accompanied by much

needed reduction in nontarget effects. Messing and Purcell (2001) have stated that in Hawai'i "the current atmosphere of bureaucracy and over-regulation is stifling the science and the practice of biocontrol to the detriment of both agriculture and native Hawaiian ecosystems." We believe the current situation could be improved by reasonably streamlining the review process rather than relaxing of regulations. Progress in biocontrol has slowed over the past few years, seemingly unnecessarily. Actual biocontrol capacity in Hawai'i appears to be declining sharply, exacerbated by late-2009 State budget cutbacks. This could critically inhibit biocontrol efforts if a serious arthropod pest of either Eucalyptus or valued dominant native plants were to establish in Hawai'i in the future.

New forest protection biosecurity regulations are needed to prevent the attack and death of valued dominant forest plants in Hawai'i, similar to that which has occurred in recent decades in the Eastern U.S. (e.g., Moser and others 2009). The impact of the guava rust (*Puccinia psidii*) disease on the invasive alien rose apple tree (*Syzygium jambos*) in Hawai'i has been a sobering reminder of the potential for major impact on a valued dominant forest plant, our related 'ōhi'a tree (Loope and La Rosa, this volume). Such effects could be permanent and might not be amenable to mitigation through classical biological control.

Accidental introductions of alien pest insects into Hawai'i or any other land mass are difficult to predict and even more difficult to prepare for. The Internet has become a powerful tool providing the potential to keep current on pests elsewhere. Most of Hawaii's pests come from California and Florida, East Asia, Australia, and the Pacific. Arguably, biosecurity in Hawai'i can potentially be "tuned" to at least reduce the probability of pests entering that will have major impacts. This principle can be applied to any subset of pest insects, including forest/forestry pests. One potentially effective method is to regulate the likely pathways that a known potential forest/forestry pest within the Pacific Rim or Florida would take to enter Hawai'i. Wood packaging material (WPM) is recognized as a notorious pathway worldwide and has received special international attention, with the desire of developing effective standardized regulations that can work for all countries' ports of entry (FAO 2002). But WPM is a small part of the problem for Hawai'i. Food commodities are certainly a significant source of pests, but pathways of the plant trade (e.g., nursery stock and cut flowers and foliage) pose by far the greatest threat for Hawai'i.

The Natural Area Reserves Commission of the State of Hawai'i, Department of Land and Natural Resources, recently developed a list of highly-valued dominant plants (Table 3), comprised exclusively of native species, in an effort to encourage the State and Federal governments to regulate imported taxa of plants closely related to those genera, thereby providing better biosecurity protection for forest resources. The Commission had been motivated to produce such a list by the arrival of three major pest threats to a valued dominant species over less than five years: *Puccinia psidii* (commonly called guava rust or eucalyptus rust), a potentially devastating pest of Hawaii's valued dominant forest tree, 'ōhi'a (*Metrosideros polymorpha*) (see Loope and La Rosa, this volume); and two insect species described above, the *Erythrina* gall wasp, (*Quadrastichus erythrinae*) and the naio thrips, (*Klambothrips myopori*). The Commission's concern was that future pests of valued dominant forest plants could seriously if not permanently damage important forest resources, plant communities, or even entire ecosystems and watersheds. We see the need to strive

toward improved biosecurity protection against new threats to valued dominant plant species that could be expanded over time to provide protection for the major components of Hawaii’s globally unique forests.

Table 3. Hawaiian valued dominant forest plants suggested by the Natural Area Reserves Commission for special quarantine protection.

Hawaiian name	Scientific name
'Ōhi'a	<i>Metrosideros polymorpha</i>
Koa	<i>Acacia koa</i>
Hapu'u	<i>Cibotium spp</i>
Mamane	<i>Sophora chrysophylla</i>
Naio	<i>Myoporum sandwicense</i>
'A'ali'i	<i>Dodonaea viscosa</i>
Wiliwili	<i>Erythrina sandwicensis</i>
'Ohelo	<i>Vaccinium spp.</i>
Pukiawe	<i>Leptecophylla tameiameia</i>
Lama	<i>Diospyros sandwicensis</i>
Hala	<i>Pandanus tectorius</i>
Naupaka	<i>Scaevola spp.</i>
Uluhe	<i>Dicranopteris linearis</i>

Nursery stock is becoming increasingly recognized as a threat by U.S. Department of Agriculture (USDA). Campbell (2008) synopsis: “There is also agreement on the reasons for the rising threat: in recent decades, plant imports have increased greatly, the geographic range of suppliers has expanded, and more rapid transport allows more pests to survive transit. At the same time, phytosanitary safeguards have been relaxed rather than tightened.” Additionally, many of the most damaging forest pathogens are unknown to science when they are introduced (Campbell 2007).

A growing coalition in Hawai'i advocates regulations prohibiting or restricting importation of close relatives, potential conduits of new pests that will otherwise inevitably degrade Hawaii’s valued dominant species and ecosystems. Hawai'i has used this method effectively for over a century to exclude pests of coffee, sugar cane, pineapple, etc. The coalition believes that an effective exclusion program to protect Hawaii’s valued dominant plant species must be instituted as quickly as possible to prevent introductions of pests, most of which are unknown or insufficiently understood. The coalition has some optimism that USDA-APHIS’ current “Quarantine 37” or “Q37” initiative may facilitate achievement of satisfactory regulations (see APHIS 2009). A list of genera proposed for regulation referred to as the NAPPRA list (Not Authorized Pending Pest Risk Analysis) will be created and become part of the USDA-PPQ Q37 regulations. A Pest Risk Analysis (PRA) would be required before importation of any plant on the list. Such Federal (and some form of complementary State) regulation of valued dominant forest plant relatives could also benefit the plant export business in Hawai'i indirectly, by keeping out generalist pests that become

established in Hawai'i and cause rejections of Hawaii's exported plants by mainland and foreign biosecurity agencies. Just as federal regulations need strengthening, the State of Hawai'i needs to build capacity to incorporate PRA methodology into its Plant Quarantine regulations, regardless of whether or not the Q37 regulations are successfully adopted by APHIS-PPQ.

CONCLUSIONS

We have here tried to summarize information on the significant forest and forestry pests established in Hawai'i. Our discussions of each are by no means thorough or definitive, but we have presented enough information on each so that this paper may serve as a "big-picture" reference on the subject.

We would argue that prior to 2005, relatively few significant forest pests became established in Hawai'i (especially so given Hawaii's notorious vulnerability to invasions), although the black twig borer and the two-spotted leafhopper stand out as inflicting very serious damage on multiple valued dominant forest species. An influx of *Eucalyptus* pests has occurred since 1993 but their impact has been modest, thanks at least in part to co-establishment of biological control agents. Two-spotted leafhopper no longer has highly damaging outbreaks, presumably because a complex of parasitoids finally took hold in most circumstances. Natural enemies or other biotic or abiotic factors have brought most of our forest/forestry pests under more or less adequate control. Black twig borer persists as a serious pest of multiple forest species.

Biological control must remain a viable option for control of widespread significant forest pests. However, both State and Federal regulation of the practice must be streamlined to speed up this inexcusably long review process, which interferes with timely mitigation of pest damage done by the target pest, and even interferes with simultaneous and subsequent biocontrol projects.

A new era may have started in 2005 with arrival of *Erythrina* gall wasp (EGW), *myoporum* thrips, and guava rust. Biocontrol success for EGW seems possible but only time will tell. Biocontrol of the *myoporum* thrips seems more complicated. Biocontrol for guava rust is not a possibility, and success on that front seems to depend entirely on keeping out new rust strains.

Hawai'i is geographically, demographically, and politically small in relation to the size of its invasive species problems. Preventing and addressing forest pest invasions requires quick, comprehensive, and coordinated response by government agencies (e.g., Hain 2006; Moser and others 2009). Yet funding is frequently limited for quarantine and early pest detection programs, and current State regulations pertaining to biological control, although well-intentioned, take an unacceptably long time for review and implementation. Unpredictable political outcomes for future funding of quarantine, early detection and biocontrol programs exacerbate the uncertainty of being able to effectively address future pest introductions. Indeed, Hawai'i's difficulties are likely to be worsened by the 2009 recession-related State budget cutbacks. We cannot assume there will always be solutions for the next unknown new

forest pest. New federal and state regulations to restrict importation of taxa of plants related to valued dominant and economic forest plants to prevent hitchhiking pests are clearly needed.

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DEVELOPING RESISTANT KOA-EARLY RESULTS, FROM DISEASE SURVEY TO SEEDLING RESISTANCE TESTING IN HAWAI'I

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ABSTRACT

A state-wide survey was conducted to determine distribution of koa wilt/dieback disease across the four main Hawaiian Islands: Kaua'i, Maui, O'ahu and Hawai'i. A total of 386 samples were taken at 46 different sites covering approximately 13,830 acres of natural and planted koa (*Acacia koa*) forest. Koa trees and seedlings infected by *F. oxysporum* were found on all of the major islands, in forest tree seedling nurseries, natural and plantation forests. From these samples more than 500 isolates of *F. oxysporum* were obtained. Of these, 123 isolates were tested for virulence on koa seedlings in controlled greenhouse inoculation tests. From isolate screening tests, 10 virulent isolates were identified for use in screening selected koa families for disease resistance. Between 2006 and 2009, more than 150 koa families were evaluated for their potential resistance to highly virulent stains of *F. oxysporum* in greenhouse tests. Most of the families came from wild populations. However, several seed sources were from survivors of family level progeny trials at HARC's Maunawili Field Station. Seedling wilting and mortality in greenhouse tests was monitored over a 90 day period. Seedling mortality among seed lots varied widely (4-100%) and averaged 61.5%. These initial results indicate that natural resistance to *F. oxysporum* is low within native koa populations.

INTRODUCTION

In Hawai'i, koa (*Acacia koa*) is a valuable tree species economically, ecologically and culturally. With significant land use change and declines in sugarcane, pineapple, and cattle production, there is an opportunity and keen interest in utilizing native koa in reforestation and restoration efforts. However, moderate to high mortality in many of the low-to moderate elevation plantings have impeded past efforts (Daehler and Dudley 2002). Koa wilt/dieback was first described in Hawai'i by Gardner (1980). He completed Koch's postulates and determined the primary cause of the disease was pathogenic strains of *F. oxysporum* which he designated f.sp. *koa*. *F. oxysporum* is a relatively common agricultural and nursery fungus, but the origin of strains virulent to koa is unknown.

Identifying and developing koa populations that are genetically resistant to virulent strains of *F. oxysporum* may be the key to successful koa restoration and reforestation (Sniezko 2006). Great differences in mortality among families in young koa field trials planted in the 1990's was the impetus for developing a seedling screening test and investigating potential genetic resistance to *F. oxysporum* (Sniezko 2003).

METHODS

Survey

A partial state-wide survey was conducted to determine distribution of koa wilt/dieback disease within commercial nurseries and field sites on the four main Hawaiian islands: Kaua'i, Maui, O'ahu and Hawai'i. Each location was geo-referenced and digital maps were developed.

For the field surveys, 52 koa trees exhibiting external signs of wilt and/or dieback disease were selected. Wood samples from symptomatic trees were collected from selected branches, portions of the main stem, and from roots. Emphasis was placed on collecting root samples, particularly fine feeder roots, since this is likely the point of infection by wilt-inducing organisms.

For the nursery section of the survey, we tried to select koa seedlings that showed symptoms of wilt. However, this was not possible at a few nurseries, which did not have any available diseased seedlings, and all their seedlings looked healthy. All samples were sent to the lab for isolation and identification of associated potentially-pathogenic *Fusarium* spp. The type of media used to produce seedlings was also recorded. Most nurseries used a peat based, soilless medium. A few small nurseries used a mix of cinder and soil, or soil in 4 inch pots. Individual nursery names were kept confidential for publication purposes, although isolation results were given to each nursery.

Roots and stem/branch sections were dissected into pieces about 5 mm in length. Randomly-selected pieces were surface sterilized in 0.525% aqueous sodium hypochlorite (10% bleach solution), rinsed in sterile water, and placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-14 days. Selected emerging fungi were transferred to potato dextrose and carnation leaf agar for identification using standard taxonomic guides. Percentages of sampled pieces colonized by particular *Fusarium* species were calculated.

Isolate Trials

One hundred and twenty-three isolates identified as *F. oxysporum* based on morphological characteristics were selected for testing in greenhouse seedling inoculation experiments. For each selected isolate, fungal inoculum was prepared using the procedures of Miles and Wilcox (1984). Perlite, an inert siliceous rock of volcanic origin commonly used in potting mixtures, was the matrix for fungal growth. A perlite growing media was prepared by adding 150 g of cornmeal moistened with 300 ml warm 1% potato dextrose agar (PDA) to 75 g of perlite. The perlite-cornmeal-PDA mixture was autoclaved at 121°C for 60 min, cooled, and

inoculated with spore suspensions of test fungi. Inoculum was incubated at about 24°C in the dark for at least 15 days. After incubation, inoculum was dried in open petri plates within a cabinet. Inoculum dried within 5-7 days and did not become contaminated with other microorganisms because the food base was completely colonized by inoculated fungal isolates. Once dry, inoculum was refrigerated until needed. Inoculum was ground to a fine powder and thoroughly mixed with commercial peat moss/perlite growing media (Sunshine Mix 4, Aggregate Plus, Sungro Horticulture, Bellevue, WA) at a concentration of 1:50 (w/w). Inoculum-growing media mixtures were placed into plastic containers (“dibble tubes” – 115 mm³) which were either new or had been previously sterilized by immersion in hot water (71°C for 5 min).

Seeds of *Acacia koa* from one family were nicked at their distal end with nail clippers to break dormancy and soaked in water for about 12 hours. Seeds were sown into flats containing a 50:50 (v/v) mixture of vermiculite (Sta-Green Horticultural Vermiculite, St. Louis MO) and perlite (RedcoII, North Hollywood, CA), periodically watered and monitored for germination. Following germination, when radicals were approximately the same length as cotyledons, seedlings were transplanted into the plastic containers containing inoculum-growing media mixtures. Following transplanting, seedlings were watered to activate inoculum. For each tested isolate, twenty four seedlings were evaluated. Twenty-four seedlings transplanted into peat/perlite growing media without fungal inoculum were included as a control.

When seedlings were considered dead (extensive wilting), they were carefully extracted from plastic containers, their roots washed thoroughly to remove adhering particles of growing media, and analyzed in the laboratory for root colonization by inoculated isolates. For this analysis, ten randomly-selected root pieces, each approximately 5 mm in length, from each seedling were surface sterilized as previously described, rinsed in sterile, distilled water, and placed on a selective agar medium. Plates were incubated as previously described and emerging fungi were compared with inoculated isolates to determine whether they were the same morphological species.

Tests ran a maximum of 90 days. Heights of seedlings surviving to the end of the tests were measured. A few surviving seedlings were sampled as described above to confirm re-isolation of the inoculated isolates.

Resistance screening trials

One hundred and fifty *Acacia koa* families were evaluated for resistance to a mixture of highly virulent isolates of *F. oxysporum*, selected from the isolate screening trials. For our purposes, a family represents seed from one mother tree. Five tests were run sequentially in 2007 and 2008. These resistance trials were similar to the isolate trials, except different koa families were challenged against the composite of virulent isolates. Five highly virulent isolates were used for the first three trials. Five more highly virulent isolates were added for the last two trials. Isolates used in resistance screening trials are listed in Table 1.

Seed from each family was prepared, germinated and transplanted as in the isolate trials. For each family tested, 24 seedlings were evaluated. Dead seedlings were processed and analyzed

in the laboratory for root colonization by inoculated isolates as in the isolate trials. Tests were run for 90 days, at which point roots from a sample of survivors were incubated to confirm reisolation of the inoculated isolates.

Table 1. Highly virulent *F. oxysporum f. sp. koeae* isolates used in resistance screening trials.

Isolate Number	Island	Host Information
1	Maui	Diseased seedling; fine roots
2	Big Island	Diseased tree; fine roots
3	Big Island	Diseased tree; fine roots
4	Big Island	Diseased tree; fine roots
5	Big Island	Diseased tree; fine roots
6 ¹	Kaua'i	Diseased tree; fine roots
7 ¹	Kaua'i	Diseased tree; outer stem
8 ¹	Kaua'i	Diseased tree; inner stem
9 ¹	Kaua'i	Diseased tree; rhizosphere soil
10 ¹	O'ahu	Seeds/young germinants;

¹ Isolates included in the last two trials only

RESULTS

Survey

F. oxysporum was found to be widely distributed throughout the sampled Hawaiian Islands, as it was present at nearly all sampled field and nursery sites (Fig. 1 and Fig. 2).

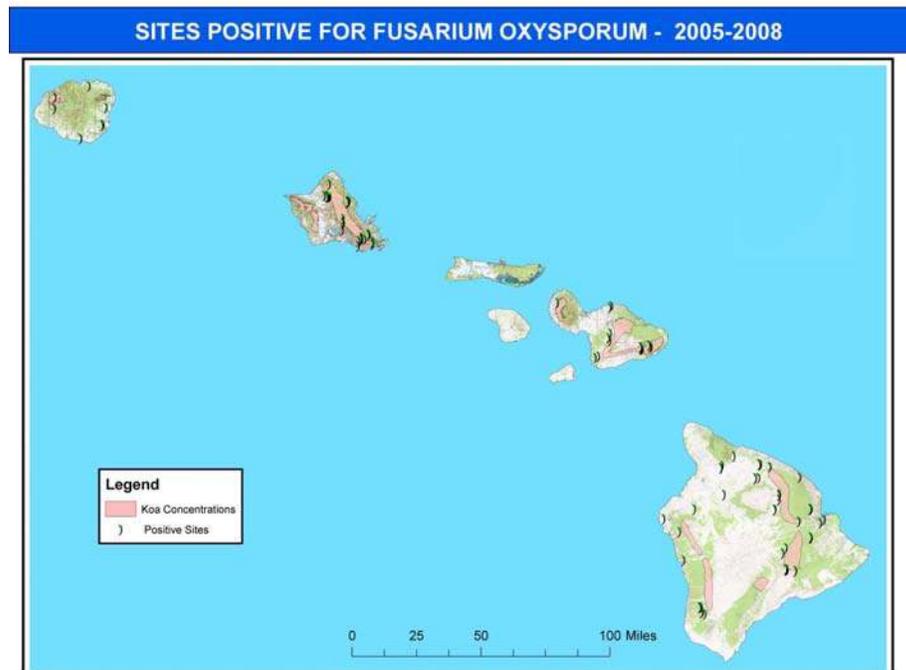


Figure 1. Sites positive for *Fusarium oxysporum*

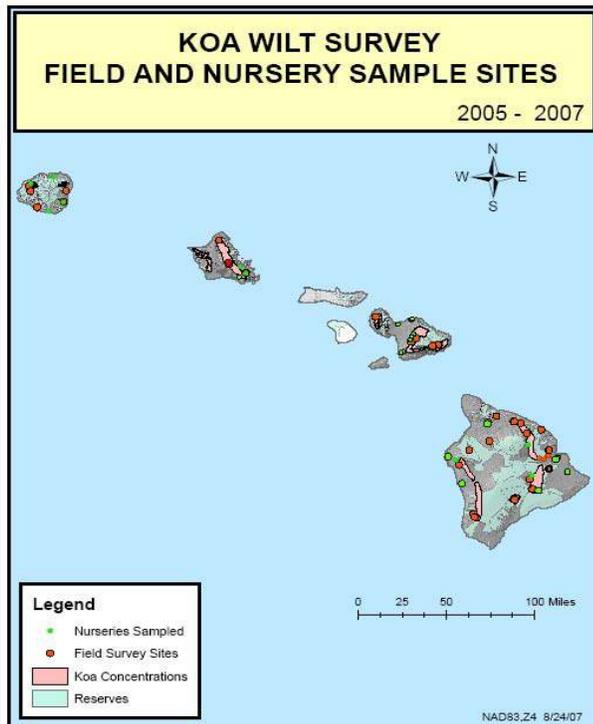


Figure 2. Field and Nursery sample locations

While several *Fusarium* species were isolated from roots of trees displaying wilt/dieback symptoms and from nursery seedlings, *F. oxysporum* was by far the most common (Table 2 and Table 3). This species was obtained from nearly half of the fine roots sampled, but was less common within larger secondary and tertiary roots. The second most commonly-isolated *Fusarium* species from roots was *F. solani*, which was found on about 10% of sampled roots.

Isolate Trials

Virulence ratings for tested *F. oxysporum* isolates were initially assigned primarily on the basis of disease production, but also included average seedling survival (number of days seedlings lived during the 90-day test) and average height of non-diseased seedlings. After the initial three trials, virulence ratings were assigned based on percent mortality after 90 days in an effort to develop a more efficient screening protocol. Virulence ratings and the proportion of isolates falling within each category are summarized in Table 4.

Table 2. *Fusarium* root colonization of diseased koa trees

	Fine Roots	Secondary Roots	Tertiary Roots	All Roots
Trees	46	33	18	18
Root Pieces Sampled	763	600	430	1793
	<u>Percent Colonization</u>			
<i>F. oxysporum</i>	44.4	29.7	23.7	34.5
<i>F. solani</i>	16.2	8.3	6.5	11.3
Other <i>Fusarium</i> spp	9.7	2.3	5.8	6.3
All <i>Fusarium</i> spp.	66.6	38.0	34.9	49.4
No Fungi	0	19.5	22.6	11.9

Table 3. *Fusarium* colonization of koa seedlings

	Roots	Stems	All Samples
Seedlings	158	62	162
Root Pieces Sampled	1892	533	2425
	<u>Percent Colonization</u>		
<i>F. oxysporum</i>	56.1	21.2	48.4
<i>F. solani</i>	9.6	8.1	9.2
<i>F. semitectum</i>	7.9	6.0	7.5
<i>F. subglutinans</i>	4.3	4.1	4.3
<i>F. equiseti</i>	1.4	3.6	1.9
<i>F. avenaceum</i>	0.1	3.6	0.8
Other <i>Fusarium</i> spp	2.8	2.6	2.8
All <i>Fusarium</i>	77.1	46.1	70.3

Table 4. Virulence classification of *Fusarium oxysporum* isolates

Virulence Rating	Percent Diseased / Mortality ¹	Number of Isolates	Percent of Isolates
Highly Virulent	> 80	9	7.1%
Moderately Virulent	50-80	25	19.8%
Low/Non-pathogenic	< 50	92	73.0%

¹Percent of inoculated seedlings that died during tests

About 27% of the tested isolates exhibited high or moderate virulence on young koa seedlings under our greenhouse inoculation conditions. Over 70% of the isolates tested exhibited low virulence or were considered non-pathogenic.

Resistance Screenings

The percentage of seedlings that survived our greenhouse inoculation trials varied greatly among families, ranging from 0-96%, with an average survival of 38.5%. Resistance ratings for individual seed sources were assigned based on seedling survival. Resistance rating categories and the proportion of families falling within each category are summarized in Table 5.

About 12% of all tested koa families exhibited a high frequency of resistance to the mixture of highly virulent *F. oxysporum* isolates under greenhouse inoculation conditions. Almost 50% of tested families were identified as susceptible because they showed low seedling survival. Less than 5% of families from wild populations were identified as resistant as compared to almost 35% of families from the Hawai'i Agriculture Research Center (HARC) plantings (Table 6 and Table 7).

Table 5. Frequency of resistance within all koa families¹ tested

Family resistance rating	Percent survival at 90 days ²	Percent of families tested
Resistant	75 or more	12.4%
Tolerant	40-75	40.4%
Susceptible	0-40	47.2%

¹A family represents seed from one mother tree

²Percent of 24 inoculated seedlings alive after 90 days

Table 6. Frequency of resistance within koa families from wild populations

Family resistance rating	Survival at 90 days ¹	Percent of families tested
Resistant	75% or more	4.5%
Tolerant	40-75%	37.8%
Susceptible	0-40%	57.6%

¹Percent of 24 inoculated seedlings alive after 90 days

Table 7. Frequency of resistance within koa families from HARC's plantings

Family resistance rating	Survival at 90 days ¹	Percent of families tested
Resistant	75% or more	34.8%
Tolerant	40-75%	47.8%
Susceptible	0-40%	17.4%

¹Percent of 24 inoculated seedlings alive after 90 days

DISCUSSION

We found that *Fusarium* spp. are common residents of koa trees exhibiting wilt/dieback disease symptoms in Hawai'i. Although several different species were detected, the most common species were *F. oxysporum* and *F. solani*. *Fusarium oxysporum* was concentrated within koa root systems, especially on fine roots. This species was widely distributed within most nurseries and on most field sites. Nursery management practices should be amended to prevent the unintentional spread of *F. oxysporum*, particularly if nursery isolates prove to be highly virulent on koa.

Protocols used to test virulence of *F. oxysporum* on young koa seedlings were developed initially for use on conifer seedlings (James 1996; James and others 1989). These procedures were adapted for use on koa seedlings to identify highly virulent isolates that could be used to screen koa families for resistance.

We found that only a small percent of tested isolates of *F. oxysporum* could be classified as highly or moderately virulent. These isolates were readily identifiable because they killed the majority of inoculated seedlings. Most seedling mortality usually began about one month after inoculation and extended for the next 3-4 weeks. All tested isolates, even those considered non-pathogenic, always infected roots of inoculated seedlings. Inoculated roots exhibited no noticeable necrosis or discoloration. i.e., they were white and appeared completely healthy. However, they were extensively colonized by inoculated isolates, even to the point where often no other fungi were detected during root assays. We suspect that non-pathogenic isolates were unable to successfully colonize vascular systems and thus spread systemically throughout inoculated seedlings (Beckman and others 1989; Nelson and others 1981). Such isolates may have been restricted to root cortical cells as endophytes, which did not adversely affect seedling health (Bloomberg 1966; Dhingra and others 2006).

There was wide variability of fungal strains within the *F. oxysporum* species complex throughout Hawai'i. Although most of these organisms appear morphologically similar, there are apparently extensive genetic differences (Yang and others 2007) that may affect pathogenicity on koa plants. We plan to expand testing to confirm proportions of fungal populations capable of eliciting koa wilt and to have more highly-virulent isolates for resistance screening trials. This will improve our confidence that selected resistant families will perform well after planting when exposed to natural populations of *F. oxysporum*.

Resistance screening trials conducted so far indicated that natural resistance to pathogenic strains of *F. oxysporum* exists within koa populations, although at low levels. Several resistant families were identified. We found survival among different koa families to be

highly variable, ranging from 0 to 96%. Survival rates were spread across a spectrum between the extremes, but few families experienced 75% survival or more.

Although results indicate that resistant families occur at a low frequency in wild koa populations, resistant families occurred more frequently within HARC's plantings, indicating that resistance can probably be improved through traditional breeding. We plan to expand our screening to include many more koa families and to validate greenhouse screening trials using field trials.

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CURRENT MOLECULAR CHARACTERIZATION AND DISEASE MANAGEMENT RESULTS FOR PUCCINIA PSIDII, THE 'ŌHI'A RUST

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ABSTRACT

The 'ōhi'a rust caused by *Puccinia psidii* remains a serious threat to the native 'ōhi'a forests in Hawai'i. Samples of infected Myrtaceae from new areas continue to be submitted to our laboratory for confirmation and storage. The rust has also been found on new hosts. In the fall of 2008, it was discovered on severely infecting allspice plants on the island of O'ahu. Single spore cultures of rust from rose apple, brush cherry, nioi (*Eugenia reinwardtiana*) and allspice were multiplied on rose apple plants to increase urediniospores for bulk spore storage. Use of single spore infections and subsequent spore multiplication is a method to obtain pure cultures of the rust. The DNA extracted from these samples represents pure culture lines from their respective hosts. Molecular analysis of the samples continues and at present, results bolster the observation that the rust infecting the various Myrtaceae hosts in Hawai'i is a single strain. Fungicide trials initiated in 2008 continue, and compounds tested are commercially available to nursery growers and the general public. Fungicides with trifloxystrobin, tebuconazole, and myclobutanil as active ingredient are promising candidates. Results of the disease survey, molecular analysis, and fungicide trials will aid state officials in generating import restrictions and protocols for shipping Myrtaceae hosts into Hawai'i.

INTRODUCTION

Puccinia psidii was first observed infecting 'ōhi'a (*Metrosideros polymorpha*) plants at a commercial nursery in Hawai'i in 2005 (Fig. 1) (Uchida and others 2006). In 2006 a statewide disease survey was initiated to determine the distribution and host range of the rust. At the same time, Dr. Shaobin Zhong of the University of Hawai'i, Manoa, developed a set of 15 microsatellite markers and began comparing the Hawai'i isolates with those from Florida and Brazil (Zhong and others 2007).

The rust continues to be found on new Myrtaceae hosts and in new area around the state as evidenced by the discovery of it infecting allspice (*Pimenta dioica*) for the first time in 2008. Samples of infected allspice (Fig. 2) from three areas on the island of O'ahu were collected and stored for molecular analysis. In 2008 single spore cultures were established for rust samples from rose apple, brush cherry, allspice, and the native Hawaiian tree nioi. Fungicide screening tests were begun in 2008 to identify possible control agents that could reduce the spread of the rust in greenhouse grown 'ōhi'a plants.



Figure 1. 'Ōhi'a (*Metrosideros polymorpha*) infected with rust.



Figure 2. *Puccinia psidii* infecting Allspice (*Pimenta dioica*).



Figure 3. Endangered Nioi (*Eugenia koolauensis*) in the native Hawaiian forest is severely affected by the rust.

METHODS

Trips to the island of Hawai'i, Maui, and Kaua'i were conducted to survey and collect samples of Myrtaceae hosts plants infected with *Puccinia psidii*, with a focus toward collecting rose apple (*Syzygium jambos*) and 'ōhi'a (*Metrosideros polymorpha*) from every island. When the rust was found, the infected plant tissue was carefully collected. Leaves were cut and placed in a paper towel and put into a 3 ½ x 6 ½ inch brown envelope. The envelope was labeled, then put into a plastic ziplock bag with desiccator beads to reduce moisture and prevent mold from forming on the tissue. After returning to the laboratory on O'ahu, the plastic bag was put in a 9 ½ x 13 x 3 inch Rubbermaid plastic storage bin and stored in a refrigerator at 5°C. In the field, if the rust infection was extremely heavy, bulk spores were collected with a Cyclone vacuum tip and stored in a gel capsule. The gel capsule was placed in a 2 ml plastic storage tube with a rubber gasket and desiccator beads on the bottom to reduce moisture. The tube was put in a – 80° C freezer for long-term storage. Samples of rust from 'ōhi'a and rose apple were collected from all four islands. Over eighty samples from various host plants were collected from O'ahu, Maui, Kaua'i, and Hawai'i.

DNA was extracted from twenty-one rust samples representing nine different Myrtaceae hosts, and a rust sample from Myrtle intercepted by the Hawai'i Department of Agriculture from a California shipment. The Lyse-N-Go Reagent (Thermo Scientific #78882) method was used to extract the DNA from all samples. The PCR reaction was used with florescent primers developed by Dr. Shaobin Zhong to amplify and label the SSR sites. A sample of *Puccinia psidii* rust DNA from Florida was included in the PCR reaction to compare the Hawai'i isolates to one from the mainland. This Florida sample was previously identified by Dr. Zhong as similar to the one in Hawai'i. Visualization was done on 2 % agarose gel with ethidium bromide. The DNA of isolates with fragments that were successfully amplified was sent to a genomics lab at the University of Hawai'i for genotype analysis.

Single spore cultures of rust were initiated to obtain pure lines for molecular analysis. Rust spores from rose apple were spread on a 10 cm water agar plate using a sterile wire loop. Single spores were identified with a stereo-microscope; then excised from the agar using a scalpel. A single spore was placed one per young leaf on a healthy rose apple plant growing in a 4-inch plastic pot. A small mark was made on the leaf using a sharpie pen to aid in identifying where the pustule should form. Only small, fleshy, young, red-purple to pink leaves were inoculated. Three plants were inoculated and were put in a clear plastic bag with 100% humidity and kept near a window for 2 weeks for pustule development. When pustules formed, spores were collected from one pustule only and spread on another plant. A soft tipped paint-brush was used to collect the spores from the pustule, and paint them onto the underside of the young leaves of the other plant. After two weeks, many pustules developed, and after one month the infection had spread and spore production was heavy. Leaf tissue covered with spores was cut out and stored in a 2 ml plastic storage tube with a rubber gasket. The tube was labeled and stored in a -80° C freezer. This procedure was used to multiply rust established from single spores from rose apple (*Syzygium jambos*), brush cherry (*Eugenia paniculatum*), allspice (*Pimenta dioica*), and the native Hawaiian plant nioi (*Eugenia reinwardtiana*).

In order to aid nursery owners in controlling the rust on 'ōhi'a seedlings in their nurseries, fungicide efficacy tests were conducted on commercially available fungicides currently registered for ornamentals. Six fungicides were tested for efficacy: Heritage[®] (azoxystrobin), Eagle[®] (myclobutanil), Cleary 3336[®] (thiophanate methyl), Compass O[®] (trifloxystrobin), Chipco[®] (iprodione), and Bayer Advanced Disease Control[®] (tebuconazole). The tests were conducted at a commercial nursery on the island of O'ahu. Fungicides were mixed at the maximum recommended rate for ornamentals, and sprayed on 'ōhi'a seedlings that were already infected with rust and growing individually in 6-inch plastic pots. Active rust colonies with bright yellow to orange pustules were present on young leaves of every plant at the start of the test. The fungicides were sprayed on the infected 'ōhi'a plants three times, at 2-week intervals. Effective fungicides prevented the rust from infecting new leaves. Disease severity ratings were taken weekly and the test was ended after 8 weeks.

RESULTS

Genomic analysis of the PCR fragments revealed that all of the Hawai'i isolates are the same, and that they are the same as the Florida and California strains. These results were obtained utilizing five SSR markers tested on nineteen Hawai'i rust samples, one Florida and one California sample (Table 1). Each labeled sample produced two peaks at the predicted fragment size indicating a heterozygote of one genotype because the rust spores have two nuclei.

Three fungicides were identified as good candidates for controlling the rust on 'ōhi'a plants already infected with the rust. Bayer Advanced Disease Control[®] with active ingredient tebuconazole, Rally[®] with active ingredient myclobutanil, and Compass O[®] with active ingredient trifloxystrobin were the best in preventing infection from reoccurring on treated 'ōhi'a plants (Table 2). After eight weeks, no new pustules formed on these treated plants and the existing pustules became dry and non infectious. All of the treated plants were in close proximity to other plants with active rust pustules that provided inoculum spores to infect new susceptible leaves. However, plants treated with these fungicides showed no signs of new infection even four weeks after the last fungicide application. In comparison, control plants maintained active pustules on previously infected leaves and on new leaves that became infected.

DISCUSSION

Rust samples from nine Myrtaceae host plants were collected from O'ahu, Maui, Kaua'i, and Hawai'i. Nineteen Hawai'i rust samples, a California and a Florida rust sample were used for genomic analysis. Although the five primers used in the genomic analysis show no difference in strains, a more robust analysis with more primers would add to the confidence level for the current results. Five additional primers have been acquired and will be tested on the twenty-two rust samples previously tested bringing the total to ten primers. If the results with the five additional primers are the same, then we can say with greater confidence that there is only one strain in Hawai'i and at present it is most severe on the rose apple.

Single spore cultures of rust from rose apple, brush cherry, nioi (*Eugenia reinwardtiana*) and allspice were successfully multiplied on rose apple plants to increase urediniospores for bulk spore storage. The DNA extracted from these samples represents pure culture lines from their respective hosts. Molecular analysis will continue as the rust is found in new areas in the state and on new infected Myrtaceae hosts.

Three effective fungicides Bayer Advanced Disease Control[®], Rally[®], and Compass O[®] were identified to help control the rust in greenhouse grown 'ōhi'a. The grower's ability to utilize different fungicides will aid in the long term control of the disease in nurseries as growers can rotate chemicals and delay the buildup of pathogen resistance. Use of fungicides in the field would have limited value as it is impractical to apply fungicides to forest trees on a regular basis. However, short term treatment of the highly endangered and highly susceptible native, endangered, Hawaiian plants such as the *Eugenia koolauensis* in the field (Fig. 3),

Table 1. Host Range Test *Puccinia psidii* from Myrtacea in Hawai'i

Host	Island	Collection Site	Sample ID	HEX Green PsSSR012	FAM Blue PsSSR018	HEX Green PpSSR136	FAM Blue PpSSR014	NED Yellow PpSSR161
1 Allspice	O'ahu	Waianae Kai	PS#86	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
2 Bottle brush	Hawai'i	Hilo	PS#61	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
3 Brush cherry	Hawai'i	Mealani	PS#65	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
4 Brush cherry	Hawai'i	Mealani Exp. Station	PS#69	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
5 Myrtle	California	Intercept	PS#64	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
6 Myrtle	Maui	Lower Kimo Road	PS#14	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
7 Myrtle	Hawai'i	Hilo, Aileen Yeh's	PS#66	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
8 'Ōhi'a	Hawai'i	IKAIKA, Nursery	PS#20	234 and 240	167 and 169	132 and 143	208 and 212	275 and 277
9 'Ōhi'a	Kaua'i	Wailua Exp. Station	PS#51	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
10 'Ōhi'a	Maui	Upper Kimo Road	PS#13	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
11 'Ōhi'a	Hawai'i	Kamuella	PS#84	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
12 Paperbark	Kaua'i	Wailua Exp. Station	PS#53	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
13 Paperbark	O'ahu	Kahuku	PS#74	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
14 Paperbark	O'ahu	Nuuanu	PS#81	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
15 Rhodomyrtus	O'ahu	Kaneohe	PS#42	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
16 Rhodomyrtus	Kaua'i	Half Way Bridge	PS#45	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
17 Rose apple	O'ahu	Manoa, Puu Pia	PS#6	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
18 Rose apple	Maui	Jeremy's house	PS#16	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
19 Rose apple	Hawai'i	IKAIKA, Nursery	PS#24	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
20 Rose apple	Kaua'i	Lawai Store	PS#48	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
21 Wax Flower	Maui	Kula Exp. Station	PS#59-5	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277
22	Florida		F01	230 and 236	167 and 169	132 and 143	208 and 212	275 and 277

may protect new leaves from becoming infected. This would give the tree time to recover its vigor, form a better defense against infection, and improve its overall ability to survive. At present, no fungicides are cleared for use in the forest, but with these positive preliminary results, a special use request can be made to the Hawai'i Department of Agriculture to allow the application of Eagle[®] and Bayer Advanced Disease Control[®] on *Eugenia koolauensis* in the forest.

Table 2. Fungicide Efficacy Test

Active ingredient	Trade Name[®]	Synonym[®]	Efficacy
Tebuconazole	Bayer Advanced Disease Control	Elite, Orius, Folicur, Tebuject, Uppercut	Very Good
Trifloxystrobin	Flint	Compass O, Trilex, Stratego	Very Good
Myclobutanil	Rally	Eagle, Laredo, Systhane	Good
Azoxystrobin	Heritage	Quadris, Amistar, Abound	Good
Thiophanate-methyl	Cleary 3336	Cavalier, Spectro, Allban, Fungo, Topsin M	Poor
Iprodione	Chipco	Rovral, Iprodione, Ipro, 26GT	Poor

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***PHYTOPHTHORA CACTORUM*, *P. RAMORUM*, *P. PLURIVORA*,
MELAMPSORIDIUM HIRATSUKANUM, *DOTHISTROMA SEPTOSPORUM* AND
CHALARA FRAXINEA, NON-NATIVE PATHOGENS IN FINLAND**

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ABSTRACT

International trade and travel increase the risk of pest spread. Climate change might also improve the establishment of introduced species into new geographical areas. The ornamental trade has shown to be an important source of alien Oomycetes, especially *Phytophthora* species.

Two other pathogens, which have recently arrived in Finland, are ascomycetes *Chalara fraxinea* and *Dothistroma septosporum*. The typical symptoms of red band needle blight were found on *Pinus sylvestris* in many locations in summer 2007 and 2008. Isolations from acervuli on needles resulted in pure cultures of *D. septosporum*, the anamorph of *Mycosphaerella pini*. First signs of ash decline were found on *Fraxinus excelsior* in 2007. Although ash is indigenous only in southern Finland, the species can be cultivated in the central region. Thus far, *C. fraxinea* has only been isolated from trees in southwest Finland and Åland archipelago.

NON-NATIVE PATHOGENS IN FINLAND

Phytophthora cactorum

In Finland, *Phytophthora cactorum* was isolated for the first time in 1990 from strawberry (*Fragaria x ananassa*) plants suffering from crown rot. A year later it was isolated from necrotic stem lesions of silver birch (*Betula pendula*) seedlings (Fig. 1) growing in forest nurseries (Lilja and others 1996, Hantula and others 1997, 2000). Since then, this imported pathogen has caused crop losses in strawberry fields mainly as an agent of crown rot and increased the culling of infected birch seedlings in forest nurseries.

The crown rot isolates from strawberry within Europe have shown to be genetically identical (Hantula and others 1997, 2000, Lilja and others 1998, Eikemo and others 2004). This suggests that it is more obvious that *P. cactorum* has spread within seedling material than due to natural dispersal. The movement of *P. cactorum* on birch stays unclear since genetic analysis has been done only with isolates from Finland (Hantula and others 1997, 2000, Lilja and others 1998, Eikemo and others 2004).

We have monitored the effect of *P. cactorum* infection on container-grown silver birch seedlings in the nursery and after out-planting (Lilja and others 1996, 2007a, Lilja and others

unpublished). In two experiments, diseased and healthy silver birch seedlings were out-planted. Each seedling was assessed using a scale of 1 to 4 where: 1= no lesion 2 = lesion < 5 mm² 3 = lesion > 5 mm² but not covering over half of the stem diameter and 4 = lesion spread over half of the stem diameter but not girdling the stem. In the nursery, stem lesions affected the growth and seedling shoot height was related to disease severity. Asymptomatic birches were taller than diseased individuals and the shortest were those with stem lesions covering over half of their stem diameter (Lilja and others 1996, 2007a, Lilja and others unpublished). Seedling height growth in reforestation areas was directly related to disease rating. The shortest seedlings with stem lesions covering over half of their stem diameter grew more than the taller seedlings (healthy controls or seedlings with smaller stem lesions). Thus, differences in shoot heights between diseased and apparently healthy seedlings in the nursery reduced dramatically but did not disappear after out-planting. However, seedling mortality increased with disease severity (Lilja and others 1996, 2007a, Lilja and others unpublished).



Figure 1. Stem lesion caused by *Phytophthora cactorum* on *Betula pendula* seedling (A. Lilja).

Phytophthora ramorum

A new species of *Phytophthora*, *P. ramorum* was described by Werres and others (2001) and found associated with a twig blight on rhododendron (*Rhododendron* spp.) and viburnum (*Viburnum* spp.) in Germany and the Netherlands. Later, the same species was found to be responsible for Sudden Oak Death (SOD) of oaks (*Quercus* spp.) and tanoaks (*Lithocarpus densiflorus*) in California (Rizzo and others 2002, 2005, Davidson and others 2005). Although the pathogen has been detected on a few trees in Europe (Brasier and others 2004), our continent has so far been spared the SOD epidemic seen in western North America.

In spring 2004, *P. ramorum* was found for the first time in Finland. It was isolated after a positive PCR-reaction from commercial rhododendron plants originating from other EU

member states (Lilja and others 2007b). In the following August, the pathogen was also isolated from rhododendrons (*Rhododendron catawbiense* and several other cultivars) produced by micropropagation in a Finnish nursery (Lilja and others 2007b). Since then, the Finnish Food Safety Authority (Evira) has carried out extensive surveys in the nursery. Today, we know that the pathogen remains in the nursery, although all rhododendron seedlings and growth media have been destroyed every year in the area where the infection has been found. Routine examinations rely on a *P. ramorum*-specific PCR and isolation from positive samples, as well as verification of morphological identification by partial β -tubulin gene sequencing (Lilja and others 2007b).

Risk analyses assume that the consequences of pest introduction are positively correlated with a pest's host range (Cave and others 2005). *P. ramorum* has many hosts in different plant families (Knight 2002, Denman and others 2005). In pathogenicity tests run by us, *P. ramorum* caused stem lesions on silver birch and common alder but Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) were resistant (Lilja and others 2007b). On English oak (*Quercus robur*), the tree species present in southern Finland, *P. ramorum* only caused minor lesions or no lesions.

Phytophthora plurivora

P. plurivora used to be included in the *P. citricola* species complex, but was recently separated from it and described as a new species (Jung & Burgess 2009). This species seems to be abundant in forests, semi-natural ecosystems and nurseries across Europe, causing bark necroses, fine root losses and dieback on at least 11 woody host species, including *Quercus robur* (Jung and Blaschke 1996), *Alnus glutinosa* (Jung and others 2005, Jung and Nechwatal 2008) and *Picea abies* (Nechwatal and Oßwald 2001). We have isolated *P. plurivora* since 2004, originally from rhododendron cultivars known to be infected with *P. ramorum* (Lilja and others 2007b). In 2007, *P. plurivora* was also isolated from *Syringa* sp. Originally, our *P. plurivora* isolates were identified as *P. inflata*. The identification was supported by the fact that the beta-tubulin sequence of *P. inflata* IMI 342898 (isolated from *Syringa vulgaris* in the UK) in the GenBank had 100 % match with our isolates. However, the species status of *P. inflata* has been changed, since the original type isolate (Caroselli and Tucker 1949) has been lost, and it is clear that the isolates identified as *P. inflata* should be re-assigned to other species of the *P. citricola* complex including *P. plurivora* (Jung and Burgess 2009). In our pathogenicity trials, *P. plurivora* was able to infect most of the tested host plants including strawberry, silver birch, common alder, grey alder, Norway spruce (Fig. 2) and lingonberry. The only resistant woody species in our trials was Scots pine.

Melampsorium hiratsukanum

In the mid-1990s, an epidemic of foliar rust on *A. glutinosa* and *A. incana* was observed in Estonia and Finland (Pöldmaa 1997, Kurkela and others 1999). The morphological similarity of the pathogen to *M. hiratsukanum* was noted and recent work confirmed the species identification (Pöldmaa 1997, Kurkela and others 1999, Hantula and others 2009). The infection causes considerable damage to *Alnus* foliage in late summer, when diseased trees can easily be seen from a distance. The whole foliage turns brown and leaf margins curl inward. Successive infections can cause tree death.



Figure 2. *Phytophthora plurivora* infection on inoculated *Picea abies* seedling (left) and a control (A. Lilja).

A comparison of urediniospore morphology of *M. hiratsukanum* found no differences among material originating from Austria, Estonia, Finland, Japan, or Switzerland (Hantula and others 2009). Furthermore, sequence analysis of the ITS region for a selection of these samples detected only minor differences and reveal *M. hiratsukanum* in East Asia and Europe to belong to a single palearctic population (Hantula and others 2009). Thus, as proposed earlier by Kurkela and Hantula (1999), it seems likely that spores arriving from East Asia caused the recent European epidemic in foliar rust on *Alnus* spp.

Dothistroma septosporum* - *Mycosphaerella pini

Red band needle blight caused by *Dothistroma septosporum* is an economically important disease causing premature defoliation. The perfect stage of *D. septosporum* is *Mycosphaerella pini* (Barnes and others 2004). The typical symptoms of red band needle blight were found on Scots pine (Fig. 3) during the summers of 2007 and 2008 in 14 rural districts of southern and central Finland (Müller and others 2009). Red bands with aggregations of conidial stromata on otherwise brown attached needles were frequently encountered on saplings and young trees in dense stands and sporadically on lower twigs of mature trees (Müller and others 2009). Disease symptoms were also observed on the needles of contorta pine (*Pinus contorta*) and cembra pine (*P. cembra*), which occur in Finland at a low frequency (Müller and others 2009).



Figure 3. Red bands caused by *Dothiostroma septosporum* on needles of *Pinus sylvestris* (M. Müller).

Pure cultures of *D. septosporum* were isolated from acervuli on needles of pine trees growing in forest conditions and their identification was verified by sequencing (Müller and others 2009). Sequences of the ITS-region (including 5.8SrRNA gene) obtained from the Finnish isolates were identical to each other and over 50 published sequences of *D. septosporum* (Müller and others 2009). In a nursery experiment, brown segments and red bands appeared on inoculated 1-year-old seedlings of Scots pine within a month and conidial stromata could be seen on needles 2 to 4 weeks after the first symptoms (Müller and others 2009). Results from the inoculation experiment and probable aerial dissemination suggest that *D. septosporum* likely occurs in Finnish nurseries although the low number of pines produced and routine application of fungicide restrict its detection and limit its establishment.

***Chalara fraxinea* - *Hymenoscyphus* sp.**

During recent years, common ash (*Fraxinus excelsior*) in Europe has shown a large-scale decline. The symptoms include 1) wilting and premature shedding of leaves, 2) necroses of leaves, buds, leaf stalks and bark, 3) top and shoot dieback, and 4) cankers on shoots, branches and stems (Fig. 4). The fungus *Chalara fraxinea* is shown to be responsible for the disease (Kowalski 2006, Kowalski and Holdenrieder 2009a, Bakys and others 2009a, b). Kowalski and Holdenrieder (2009b) have suggested that the teleomorph of *C. fraxinea* is *Hymenoscyphus albidus*, an ascomycete, which has been known in Europe for a long time and is considered a harmless decomposer of fallen leaves (Ellis and Ellis 1997). However, some recent studies have found certain genetic differences among

Hymenoscyphus isolates in areas devastated by the disease, as compared with areas where the disease has not yet been observed (H. Solheim and B. Marcais unpublished).



Figure 4. *Chalara fraxinea* infection on achene of *Fraxinus excelsior* (A. Lilja).

In Finland, the first signs of ash decline were seen in 2007. Although ash is native only in southern Finland, the species can be cultivated in the central region. Thus far, *C. fraxinea* has only been isolated from trees growing in their native range in southwest Finland and Åland archipelago (Rytönen and others 2010).

The teleomorph is wind-transmitted and likely more important to dispersal than the sticky conidia of *C. fraxinea* (Kowalski and Holdenrieder 2009). *C. fraxinea* may be able to disperse in plants or wood. Although insect vectors are important in the dispersal of several species of *Chalara* (Nag Raj and Kendrick 1993), none have been identified for *C. fraxinea*. However, the symptoms of infection include, and are often similar to, those seen on trees infested by the emerald ash borer (*Agrilus planipennis*) in North American ash.

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BIOLOGY AND MANAGEMENT OF GALL RUST DISEASE CAUSED BY *UROMYCLADIUM TEPPERIANUM* ON *FALCATARIA MOLUCCANA* IN INDONESIA NURSERY

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ABSTRACT

Batai (*Falcataria moluccana* (Miq.) Barneby and J.W. Grimes) is one of the valuable multipurpose tree species for forest plantations in Indonesia. Since 1993, a gall rust disease, caused by *Uromycladium tepperianum*, has been identified as a dangerous malady of batai resulting in severe damage to all growth stages of the plant, particularly on seedlings in the nursery. Affected seedlings usually lose their leaves and becoming stunted, eventually dying with disease incidence in the nurseries of 90 to 100 percent (De Guzman and others 1991).

U. tepperianum only produced teliospores and no alternate hosts were involved in its life cycle. The fungus was observed to produce teliospores 7 days after inoculation. The teliospore germinates 10 hours after inoculation to produce a basidiospore on the host surface, then a penetration peg forms six hours later and enters the host cells directly through the epidermis (Rahayu 2007). The infected cells shows hypertrophy and hyperplasia resulting in gall formation. Early detection of infected seedlings under nursery conditions is one of the principal methods of control. Infected seedlings should be removed from the nursery and destroyed. The use of fungicide is an important component of gall rust disease management program in nurseries. Fungicides have to be applied as soon as the seed germinates or the seedling is placed in the nursery.

INTRODUCTION

Gall rust disease of *Falcataria moluccana* (batai, sengon) caused by *Uromycladium tepperianum* (Sacc.) (Rahayu and others 2005) is a devastating disease; damaging and killing seedlings in nurseries and trees in plantations. This disease has been detected in the South East Asian region, such as in the Philippines (Braza 1997), Sabah in Malaysia (Lee 2004, Rahayu, 2008) and some islands in Indonesia (Rahayu 2007). The disease causes the development of chocolate brown, cauliflower-like or whip-like galls on the stem, branch, petiole, shoot and pod. Affected plant parts and severely infected *F. moluccana* trees die prematurely (Old and Cristovao 2003).

Seedlings in the nursery are one of the most susceptible stages to infection by the gall rust fungus. Affected seedlings usually lose their leaves and becoming stunted, eventually dying with disease incidence of 90 to 100% in the nurseries (De Guzman and others 1991). Twenty days infection by the gall rust fungus on the stem, shoot, and leaf stalk of 2-month-old seedlings caused 82%, 70% and 73% mortality of the seedlings, respectively (Rahayu and

others 2006). In order to determine a management strategy in the nursery, understanding the biology of *U. tepperianum*, including mode of infection, life cycle and histology of infected cells, is needed.

METHODS

The variation of symptoms expressed on the stem, branch and shoot of seedlings affected by gall rust disease was examined and photographed using Nikon digital Camera F 70. Details of gall rust disease symptoms described on seedlings are based on natural and artificial inoculations in the nursery. In order to ascertain the infection point, process and development of the gall rust fungus on batai cells, fresh infected seedlings obtained from artificial inoculation were assessed. The infection process was also assessed on seedlings in the nursery. Samples of fresh infected seedlings were kept in clear plastic bags. In order to maintain a fresh condition, samples were kept in the refrigerator at below 15°C. In order to get the best sections, samples were processed no later than 2 days after collection. No special treatment was applied before sectioning. A series of sections were carried out on 2 to 8 weeks-old seedlings from the infected nursery. Stems of seedlings with fresh gall rust symptoms were cut to about 1 cm length and inserted into styrofoam, then sectioned into 25 to 35 µm thickness with a microtome. The sections were stained with 0.25% lactophenol cotton blue prepared in tap water, a differential stain for hyphae. The stain was left for approximately 2 minutes and sections were then floated on water for approximately 1 minute to remove excess stain. As a basis for comparison, and to help with the identification of tissues in the gall, sections of uninfected stems were made. The infected cells were examined using a compound light microscope (Leica Qwin DMRB), while morphology of galls on the stem surfaces were examined using a stereo light microscope (Leica Qwin MZ8).

RESULTS AND DISCUSSION

Gall Rust Disease Symptoms in the Nursery

The earliest symptom of gall rust disease appeared on the stems and shoots of seedlings. Slightly bent shoots or stems with or without dark red necrotic lesion (Fig. 1A) are the most typical symptom. When environmental conditions were favorable for gall rust disease development, the lesions enlarged forming reddish brown necrotic spots, or a white stripe along the stem with white pustules. At this stage, teliospores may or may not form on the stem surface. Galls on 1 to 2-month-old seedlings generally form in the middle of the stem or near a branch (Fig. 1B); however, sometimes galls form near the stem base of 1-month-old or younger seedlings without apparent symptom development.

Mode of Infection

Under nursery conditions teliospores (Fig. 2A) of *U. tepperianum* cannot infect the host; they have to germinate to produce basidiospores, which usually occurs about 10 hours after inoculation. Under favorable conditions a penetration peg is formed by the basidiospore 16 hours after inoculation (Fig. 2B). The penetration peg pierces the host cell directly through the epidermis.



Figure 1. Gall rust symptoms on *F. moluccana* seedling: A) initial symptom as slightly bent on shoot, and B) young gall with reddish brown teliospores on the surface.

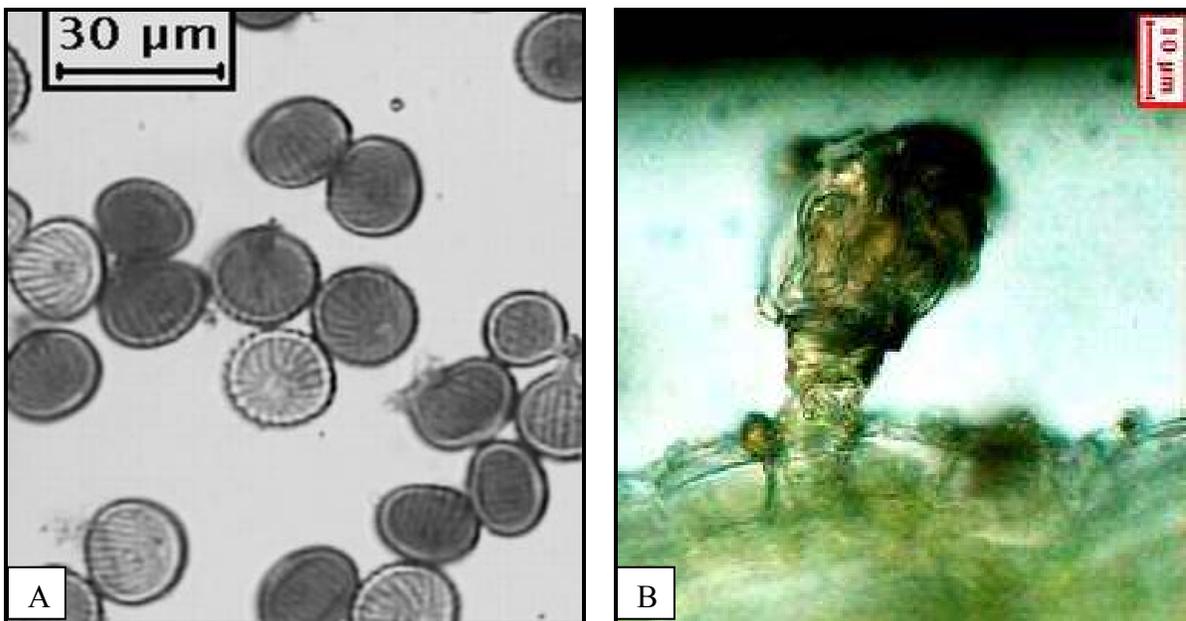


Figure 2. A) Teliospores of *Uromycladium tepperianum*, the rust fungus causing gall rust disease of *F. moluccana*, and B) basidiospore with penetration peg for direct penetration into the epidermis.

Anatomy and Histology of Infected Cells

After successful penetration of the epidermis cells by *U. tepperianum*, hyphae develop intensively in the epidermis, periderm, phloem and xylem host cells. The mycelia then destroy the cortical cell walls, causing the periderm and phloem cells to become misshapen (Fig. 3A). Sometimes, giant cells were present and surrounded by well-developed vascular bundles. Hence, there was also formation of circular vessel in the parenchyma. In the xylem, the fungus forms intracellular haustoria (Fig. 3B), intercellular hyphae within the cell walls and develop haustorial mother cells (hmc). Generally, the hypersensitive cells with haustorial mother cells die 2 days after inoculation. Some structural defense, such as the formation of papillae in the vessels and tyloses in parenchyma cells were observed as a response to infection by the gall rust fungus. Generally, 7 day after inoculation (DAI) the vegetative mycelia form pycnia, which presses against the epidermis from inside the stem until breaking through. Minute, dark brown pycnia are scattered over the galls and swellings, which bear telia that produce the teliospores. The fungus completes its life cycle on one host with no need for alternate hosts and is therefore a microcyclic rust species.

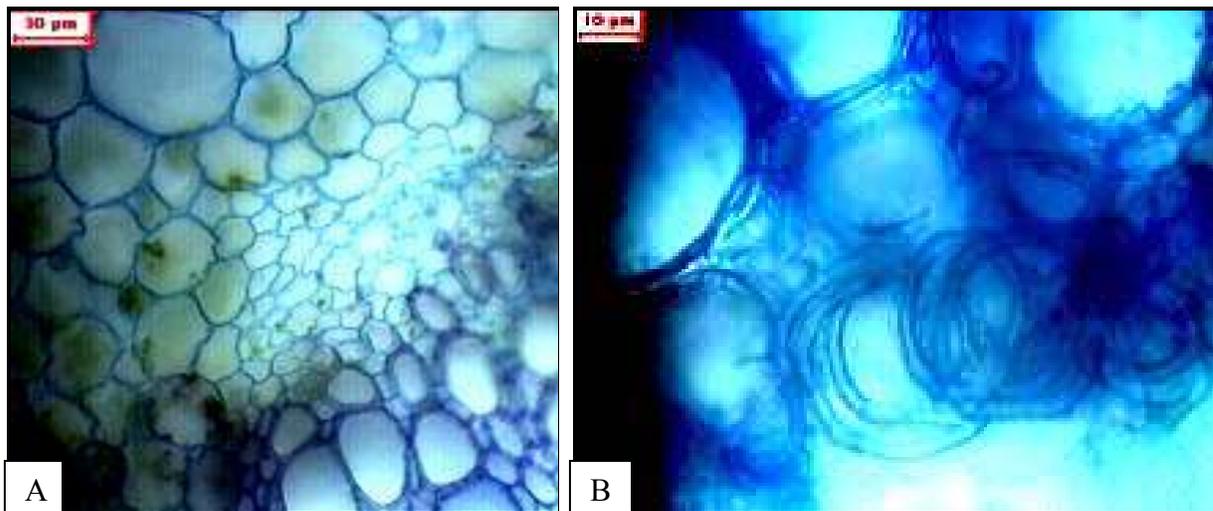


Figure 3. Development of mycelia in *F. moluccana* cells after penetration stage. A) misshapen periderm and phloem cells due to intensive growth of rust mycelia, and B) extensive intercellular haustoria between cells.

Management of Gall Rust Disease in the Nursery

Since the impact of gall rust disease on *F. moluccana* seedlings in the nursery can be severe, integrated management of gall rust disease under nursery conditions is essential.

Management strategies proposed for the control of gall rust disease on *F. moluccana* comprise of preventive measures and use of chemical control. Preventive measures include use of resistant genetic material and early detection in the nursery. The selection and breeding of resistant trees should be a long-term goal in the control of gall rust disease.

Previous gall rust disease screening showed that seedlings from Wamena, Irian and Jaya seed sources appear to be moderately resistant (Rahayu and others 2009). These seed sources may be better used for nursery production as well as breeding material for disease resistance in the future.

Early detection of infected seedlings under nursery conditions is one of the principal methods of control of gall rust disease on *F. moluccana*. As the initial symptoms on the seedling are indistinct and easily overlooked, careful observation of the seedlings should be made. Workers should be given special training on the identification of gall rust symptoms. Infected seedlings should be removed from the nursery and destroyed. Monitoring is a critical component of an effective integrated disease management program. Monitoring can be made directly through observation or by recording environmental conditions which affect disease development. Regular monitoring of gall rust disease in the nursery is important. Assessment of changes in the incidence and severity of gall rust disease should be done regularly over time. Thus, gall rust disease monitoring should be seen as an on-going commitment in any *F. moluccana* nursery program.

The use of fungicides is an important component of gall rust disease management program in nurseries. As *U. tepperianum* directly penetrates the epidermis of *F. moluccana* seedlings, regular spraying with fungicide may be used to inhibit either germination of teliospores or the infection process. In order to be effective, fungicides with copper compounds as active material, such as mancozeb, need to be sprayed or applied on the leaf (or plant) surface prior to pathogen infection. Systemic fungicides may stop an infection after it starts and prevent further gall rust disease development. Under nursery conditions, the teliospores are able to germinate about 10 hours after inoculation, thus fungicides have to be applied as soon as the seed germinates or the seedling is placed in the nursery. In addition, regular spraying has to be done every six days, starting from the first day when the seedlings were placed in the nursery, since *U. tepperianum* sporulates in seven days after inoculation. The application of chemicals for control of gall rust disease is only feasible in the nursery and is not recommended in the field.

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A SENTINEL PLANT NETWORK TO HELP ADDRESS THE PLANTS FOR PLANTING PEST PATHWAY

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ABSTRACT

More forest pests are spread by plants for planting than any other pathway. Phytosanitary protections for many countries are based on “black lists” and inspections. These strategies rely on the assumption that we know what pests exist, yet forest pests are rarely predicted in advance. International organizations such as IUFRO should promote pest discovery and information sharing systems to inform pest prevention activities. One way to develop better pest lists would be to develop a sentinel plant network. A sentinel plant network could inform our regulatory systems by capitalizing on the experience of plants exposed 24 hours every day to pests in foreign environments. Botanic gardens and arboreta already monitor their collections and diagnose plant disease and insect pests. Diagnostic support should be provided to help them identify unknown pests. The information on what is being found needs to be made available in an organized fashion. Several countries have recently initiated various forms of monitoring for pests of expatriate plants. Nursery pest professionals are asked to help diagnose new pests, and to report pest finds to their National Plant Protection Organization. Efforts, opportunities and obstacles to developing and sharing data on pests are described.

IDENTIFYING THE PROBLEM

Many forest pests, both insects and pathogens, have entered new lands via plants for planting (i.e., nursery stock). The IUFRO Work Unit 7.03.12 on Alien Invasive Species and International Trade recently endorsed a concept paper reviewing the evidence supporting strong regulatory control of this key pathway.

The World Trade Organization’s Sanitary and Phytosanitary Agreement requires that nations not limit trade except to prevent known potential pests. Therefore, science needs to do a better job of identifying pest threats before they become established in new lands. To identify pests of concern in the plants for planting pathway, a sentinel plant network is needed.

Most countries rely on a “black list” system of pest prevention. Hosts of regulated or quarantine pests are either prohibited or require some mitigation measures that reduce pest risk. Nursery plants that are not black listed are permitted to enter. This type of system assumes that we know what pests might be associated with hosts in trade, but evidence

suggests that fewer than 7% of the world's fungi are known to science (Crous and Groenewald 2005).

In the United States, inspection provides an additional safeguard. All plants for planting must enter through a port with a plant inspection station, and 75% of plant imports enter through the port of Miami. About 35 inspectors examine the 2.5 billion plants per year that enter through the Miami plant inspection station, which suggests that resources are already stretched far beyond capacity. While grapevines and fruit trees require quarantine periods under controlled conditions under the auspices of the National Clean Plant Network (<http://groups.ucanr.org/ncpn/>), most other plants do not. Some of the more risky species are allowed to be “quarantined” in the back corner of a grower's production field.

Australia and New Zealand are more risk-averse. These two countries use a “white list” system, where plants are not permitted to enter unless pest risk assessments judge them to be safe. While this approach is more limiting of trade, it is also more limiting of pests.

There is currently no international phytosanitary standard for nursery stock. Two expert working groups have developed drafts for the International Plant Protection Convention (IPPC), but so far neither draft has been circulated for country comments.

The North American Plant Protection Organization adopted a standard for plants for planting (RSPM-24) in October 2005, which calls for a systems approach to clean stock production, with chain of custody documentation to attest to proper handling throughout the production chain (NAPPO 2005). To date neither Canada, Mexico or the United States have implemented this standard, largely because doing so requires that they adopt the same standards for internal production as they require of their trading partners. Much work remains before this can be accomplished.

To further the implementation of RSPM-24, researchers and the nursery industry are working to develop best management practices and critical control point monitoring systems. Mitigations will be designed to reduce particular pests to acceptable levels. “Poster pests” will be tested to ensure mitigation measure efficacy. If the poster pests consist of a broad biological spectrum, one can assume that the mitigations will also greatly reduce the incidence of unknown pests as well.

IDENTIFYING PESTS OF CONCERN

Global botanical garden and arboreta collections are a unique and largely untapped asset, which expose non-native plants to native pests every day. The data from this giant ongoing “experiment” need to be collected and shared, so that countries can ensure that similar pests do not move in the plant trade. This information could inform prevention activities, as well as enhancing early detection of new pest arrivals.

Three components are needed to make an effective sentinel plant network:

1. Improved communication with, and better diagnostic support for, botanical gardens and arboreta worldwide.
2. Better pest reporting systems that include native pests.
3. Follow-through that mitigates newly discovered pests before they spread.

IMPROVING COMMUNICATION AND DIAGNOSTIC SUPPORT

Staff at botanical gardens already watch over their collections and examine any ailing plants. However, plant failure is often attributed to poor adaptation to local climate, without benefit of a professional diagnosis. The American Public Gardens Association recently received funding from the United States Department of Agriculture (USDA) to develop training materials for garden staff to help them identify pest problems, and know where to go for advice if unusual problems occur. They will also develop outreach materials for gardens to deliver to the plant-loving public about invasive pest impacts and the need for citizen monitoring.

In the United States, National Plant Diagnostic Network (NPDN) labs are willing to help identify pests, and direct clientele to experts who can provide management strategies. If the pest is exotic, we will “catch” the next chestnut blight before it gets thoroughly established in the US. Or if the problem is endemic to the garden’s region, and not known in the country of origin for the host plant, counterparts overseas will be grateful for an advance warning that such a pest exists. If hosts are moving in trade, they will take steps to ensure that such exchanges are from clean stock only.

BETTER PEST REPORTING SYSTEMS

Pest reporting is a requirement for members of the IPPC. Much pest data is available on the internet, but it is scattered, and largely not searchable by host plant. Countries wishing to assess risks and individuals trying to identify a pest problem need better access to information on what pests are already known, what they look like, their biology and impacts. Ideally a single data portal managed by the Secretariat of the IPPC or the United Nations Food and Agriculture Organization would provide free access to complete pest lists (sortable by host) for all countries. At the top level, information should be simply written, with photographs suitable for use by the general public. Links to more technical information, e.g., identification keys and molecular data resources are recommended.

Many botanic gardens and arboreta are working to place their collection catalogs on the worldwide web. For example, the “PlantSearch” database maintained by Botanic Gardens Conservation International (BGCI) currently contains 575,000 records of taxa found in botanic garden collections around the world (http://www.bgci.org/plant_search.php). Most gardens retain information on the sources of plants in their collection equivalent at least to what would be found on a herbarium specimen label. The USDA recently funded BGCI to grow this database, and to encourage gardens to include information on weediness of plants

in their unique environment. This information can be used by all countries to evaluate risk from the plants themselves as pests.

MITIGATE NEWLY DISCOVERED PESTS BEFORE THEY SPREAD

In the United States, if a new pest is detected, NPDN labs have a reporting system with the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) to initiate a response. A New Pest Advisory Group assesses the risk and makes a recommendation about whether or not emergency actions, such as delimitation surveys and eradication programs, are needed. Counterparts overseas are often contacted to provide information about how to best mitigate the problem.

If a domestic pest is discovered attacking an exotic plant, counterparts overseas should also be notified, so that they can assess the risk the pest poses. Because of the trade implications, there may exist some disincentive to expend resources on this, but under the IPPC agreements we have an obligation to do so. If all countries would adopt the "Golden Rule" in this regard (Do unto others as you would have them do unto you), pest prevention efforts could improve substantially. At present, this vision is just a Utopian dream.

We are witnessing the bare beginnings of a sentinel plant network of a different kind. Several countries have initiated monitoring efforts in sentinel plantings with specific trading partners. While these individual efforts are not linked, they offer good examples of different approaches to getting better pest information. INRA (Institut National de la Recherche Agronomique) has sent hundreds of seedlings of European plants to China, to establish replicated plots of sentinel plants. Kenis of CABI Switzerland is collaborating with Russian scientists to survey for insects attacking existing European plants in Russian arboreta (Kenis and others 2009). And New Zealand recently completed a comprehensive five year pilot study (Fagan and others 2008).

The botanical gardens organizations recently funded by USDA will develop training and outreach programs useful in other countries as well as in the United States. It is vital that we secure cooperation from gardens and pest specialists everywhere to reap the full benefit of this opportunity to inform the pest prevention process with scientific information on what pests exist where, and what they will eat when given the chance.

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CULTURE PRACTICE AND DISEASES OF FINNISH FOREST NURSERIES

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ABSTRACT

In Finnish forest nurseries, 99% of seedlings of Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and European silver birch (*Betula pendula*) are grown in containers in plastic-covered greenhouses. The main diseases of conifer seedlings are Scleroderris canker (*Gremmeniella abietina*), snow blights (*Herpotrichia juniperi* and *Phacidium infestans*) and needle casts (*Lophodermium seeditosum* and *Meria laricis*). Recently, Scleroderris canker has become a problem in Norway spruce, the most common species produced by Finnish nurseries since 2000. Root dieback (uninucleate *Rhizoctonia* sp.) in container-grown spruce and pine was a problem in the 1990s. Today, the disease has become less common in modern nurseries due to improvements in hygiene and cultivation practice. Birch stem lesions (*Phytophthora cactorum*) and birch rust (*Melampsorium betulinum*) have also been a problem, but are among the diseases successfully controlled with integrated pest management. Seedlings stored over winter must be routinely sprayed in order to combat grey mould (*Botrytis cinerea*). Fungal pathogens can also infect seedlings undergoing short-day (SD) treatments necessary for summer or autumn plantings and prior to freezing. Nurserymen are encouraged to use cultural and integrated pest management techniques that focus on improved hygiene such as hot water washing of containers, work surfaces and tools, as well as removing plant debris and diseased seedlings and trees around the nursery.

INTRODUCTION

Fifty percent of the Finnish tree flora is Scots pine (*Pinus sylvestris*.), 30% is Norway spruce (*Picea abies*), and several (mostly *Betula* spp.) broad-leaved species represent the rest (Finnish Statistical Yearbook of forestry 2008). However, in contrast to these proportions, most seedlings delivered from nurseries to reforestation sites in 2007 were of Norway spruce (66%; 168 million), followed by Scots pine (31%), silver birch (*Betula pendula*) (2%), downy birch (*B. pubescens*) (0.02%) and other tree species (0.98%), the most important of which were Siberian larch (*Larix sibirica*), lodgepole pine (*Pinus contorta*), Carelian curly birch (*B. pendula* var. *carelica*) and black spruce (*Picea mariana*). Most diseases known to cause problems in forestry species are fungal. In container production, the use of constant-temperature greenhouses, selected seed, pathogen-free growth medium, and carefully controlled irrigation and fertilization results in good seedling growth but may also provide ideal conditions for many biotic or abiotic diseases. Modern nurseries must optimize culture conditions to maximize seedling production while minimizing the risk of a disease outbreak. In addition to culture conditions, abiotic stress caused by environmental conditions, e.g.,

frost, or injury can also expose rapidly growing seedlings to fungal attack. In this article we give a short introduction on container tree nursery management, major diseases in Finland and collate the results on recent studies on pathogens.

SEEDLING PROPAGATION AND NURSERIES

99% of seedlings are grown in containers, germinated in greenhouses and later transferred outdoors (Finnish Statistical Yearbook of Forestry 2008). Modern container trays are composed of hard plastic cells with air slits at regular intervals on the sides to stimulate natural air pruning of roots around the periphery of the root plug. Most seedlings are cultured in densities of 400-900 m⁻² (conifers) or 150-400 m⁻² (hardwoods), depending on seedling size and age. Container cells are filled with medium-texture, low-humified Sphagnum peat that is often supplemented with dolomite lime and a base fertilizer. Seeds are sown with an automatic seeder and growth medium covered with a thin mulch of vermiculate, sand or sawdust. The pH of the growth medium is 4.5-5.1 (Rikala and Heiskanen 1995). Fertilizers are provided as fertigation according to the electrical conductivity press-water extracts from the growth medium (0.8-1.5 mS cm⁻¹) depending on the seedling developmental stage (Juntunen and Rikala 2001) and watered with mobile irrigation booms or fixed overhead sprinklers (Tervo 1999, Poteri 2003), according to container weight (peat moisture between 40-50%; V/V). The first crop of one-year-old Norway spruce seedlings is started in heated greenhouses under photoperiodic lighting in March or April and moved outdoors three months later. The second crop (two-year-old seedlings) is kept inside until the beginning of October and then moved outdoors where they remain for winter and the second growing season. Scots pine seedlings are sown in May and seedlings remain in the greenhouse until July when they are moved to a hardening site. Birch seedlings are cultured similar to the one-year-old Norway spruce crop.

Seedlings to be planted in late summer or autumn are normally short-day (SD) treated to speed the hardening process and improve the extent to which they withstand autumn frosts (Kohmann and Johnsen 2007, Luoranen and others 2007). Seedlings stored in freezer storage or outdoors might also benefit from SD-treatment by extending the storage window and/or avoiding irrigation during frost. The 2-3 week treatment consists of placing the seedlings behind blackout curtain to shorten the photoperiod to 12-14 h from 16-20 h during the growing season. Treatments are conducted from late June to the beginning of August depending on planting time and the photoperiod adjustment takes into account the seedling origin.

Approximately half of the seedlings to be delivered in spring are removed from containers in the autumn and packed in cardboard boxes. Pallets of stacked boxes are often wrapped with a thin plastic sheet to protect seedlings from drying and are stored at -1 to -4 °C for 6-7 months. The other seedlings are left to overwinter outdoors where snow cover may be thin or even absent during some winters in southern Finland. Because snow cover provides seedlings with some protection, some nurseries use snow machines to supplement the snowpack. By storing seedlings in freezers, risks related to outdoor storage are avoided and the nursery and forester enjoy greater flexibility in delivery time in spring.

DISEASES

Scleroderris canker

Recently, a new type of disease similar to Scleroderris canker, caused by *Gremmeniella abietina*, was found on nursery grown Norway spruce seedlings in Norway and Finland (Børja and others 2006, Petäistö 2008). Symptoms were similar to those observed on Scots pine; infected needles browned from the base and eventually dropped from the seedling. Necrotic lesions were also present on Norway spruce shoots (Børja and others 2006). In inoculation trials, Norway spruce needles first began browning in the mid section of the shoot, whereas in pine browning began from the top of the shoot (Petäistö 2008). The top needles of Scots pine are typically arranged in an umbrella-like form (Poteri 2008). During their first growing season, Scots pine seedlings are most susceptible to infection when buds are forming (Petäistö 1999). Second-year seedlings, however, have a higher infection risk during the period of active shoot growth (Petäistö and Laine 1999). Similarly, the vulnerable period on spruce begins later in first-year seedlings than in second-year seedlings (Petäistö 2008). During humid conditions, a peak in conidiospore release has been noted in June-July, although conidiospores may be present in the air throughout the summer months (Petäistö and Heinonen 2003).

Winter storage temperatures also influence infection rate in Scots pine seedlings; those overwintering at -7 °C and -3 °C were more diseased than those kept at 0 °C (Petäistö and Laine 1999). Seedlings stored at lower temperatures broke bud later than those kept warmer, suggesting that a physiological factor related to growth initiation may increase vulnerability (Petäistö and Laine 1999).

Infected seedlings usually appear green and healthy immediately after the snow has melted. Development of symptoms depends on weather conditions and may take a number of weeks to manifest. Control of Scleroderris canker requires repeated spray application of propiconazole (250 g/L), a triazole fungicide that has protective, curative and systemic activity. An alternative treatment is to use a mixture of prochloraz (400 g/L) and propiconazole (90 g/L).

Snow blights

Snow blights of conifers are caused mainly by *Phacidium infestans* and *Herpotrichia juniperi*. The black snow mold (*H. juniperi*) infects Norway spruce and junipers (*Juniperus* spp.), whereas *P. infestans* can infect conifers generally (Björkman 1948, Kujala 1950, Roll-Hansen 1987) and especially *Pinus* in northern parts of Europe and Asia where snow cover is deep and prolonged. Recent studies of Finnish nurseries and inoculation trials suggest that *P. infestans* also infects container seedlings of Norway spruce (Petäistö and Hantula 2009). Needles infected by *P. infestans* become yellow to red-brown following snow melt, whereas *H. juniperi* is characterized by a dark mycelium that covers and binds needles and shoots together.

Snow blights can cause considerable damage on overwintered plants in northern latitudes and at high altitudes in the south where there is sufficient snow cover during winter (Jamalainen 1956a,b, 1961). However, Petäistö and Hantula (2009) suggested that *P. infestans* was not dependent on snow cover and seedlings stored in freezers were also vulnerable to infection. Furthermore, *H. juniperi* infections in Estonia have been recorded up to a height of three meters in Norway spruce in dense forest stands during moist winter conditions where only a few inches of snow covered the ground (Hanso and Törva 1975).

Effective control of snow blights in nurseries requires spraying with fungicides such as propiconazole (250 g/L) or a mixture of prochloraz (400 g/L) and propiconazole (90 g/L). Seedlings overwintered outdoors should be sprayed as late as possible in the autumn before the formation of permanent snow cover. As the onset of winter can be unpredictable, treatment may have to be repeated several times.

Needle casts

Needle casts caused by *Lophodermium seeditiosum* and *Meria laricis* are problematic on nursery seedlings of Scots pine and Siberian larch, respectively. Both diseases are widely distributed throughout Europe and cause discoloration and browning of needles that later fall from the tree. However, seedlings infected with *L. seeditiosum* may go undetected as they can appear healthy when planted in the early spring. Typically, infected seedlings do not survive planting stress (Lilja 1986) so there is a need to identify latent infections in material stored below 0 °C prior to planting.

L. seeditiosum preferentially infects green primary and secondary first-year needles via ascospores (Lazarev 1981, Minter 1981a, b). Infection with *M. laricis* occurs during the early part of the growth period in spring while succulent foliage is present on second year seedlings. Both pathogens overwinter in fallen needles. Since infections occur during wet periods, seedlings can escape infection if dry conditions prevail during spore release. The ascocarps of *L. seeditiosum* open during wet periods between June and October (Hanso 1968, Kurkela 1979). In Finland, the only registered chemical for control of needle casts is azoxystrobin (250g/L), a wide spectrum fungicide based on naturally occurring substances found in certain species of wild mushrooms.

Root dieback

Typical symptoms for root dieback disease are stunted growth of shoots and roots and the patchy occurrence of diseased plants (Venn and others 1986, Lilja 1994, Børja and others 2006, Hietala 1995, 1997). The fungal root flora of seedlings suffering from root dieback includes at a minimum, species of *Pythium* and uninucleate *Rhizoctonia* (Galaaen and Venn 1979, Unestam and others 1989, Lilja and others 1992, Lilja 1994). In inoculation experiments, both uninucleate *Rhizoctonia* sp. and *Pythium* spp. caused damping off-like disease on seedlings younger than 5-6 weeks in which the root system is still only sparsely developed. In older seedlings only *Rhizoctonia* sp. spreads throughout the root system where hyphae can be observed on the surface of lateral root tips and inside cortical cells in the main root (Hietala 1995, 1997).

It has been hypothesized that root dieback is a disease of successive infections. Infection with uninucleate *Rhizoctonia* sp. is often initially detected with wet growth medium, because the

infected seedlings cannot fully utilize the irrigation water. Wet conditions promote secondary attack by *Pythium* spp. or other saprophytic species (Unestam and others 1989, Lilja 1994).

Seedlings occasionally suffer root dieback without conspicuous symptoms, especially when infected with uninucleate *Rhizoctonia* sp. but without any secondary infection. Seedlings in this category are shorter than disease-free stock but otherwise appear healthy. Infected seedlings should always be culled since they either suffer increased mortality or reduced growth rates after outplanting (Lilja and Rikala 2000). Today it is possible to detect the disease in planting material using a DNA based test. One benefit of this test is its higher sensitivity compared to traditional isolation (Hantula and others 2002).

Root dieback has become less common in modern nurseries due to improvements in hygiene (Iivonen and others 1996, Kohmann and Børja 2002), cultivation practice and targeted treatments, i.e., immersion in 80 °C water baths for 1 minute kills *Rhizoctonia sclerotia* (Iivonen and others 1996). One of the most important factors in modern cultivation practice is that containers are supported on racks during the growing season, which improves ventilation and drainage and thus prevents waterlogging of roots; a problem known to increase the incidence of root dieback (Venn and others 1986, Unestam and others 1989, Lilja and others 1998).

Stem lesions and top dying of birch

In Finland, necrotic stem lesions and top dying have been a severe problem in container-grown silver birch seedlings (Lilja and others 1996, Juntunen 2000). In 1991, *Phytophthora cactorum* was isolated from lesions and inoculations resulted in symptoms identical to those seen on birch seedlings in nurseries (Lilja and others 1996, Hantula and others 1997, 2000). Symptoms caused by *P. cactorum* vary according to seedling development. Following heavy rains in late June, the first lesions often appear and seedlings can suffer top dying. Seedlings infected at an early or succulent stage often die. When the bark is more suberized, lesions are more often born at the base of stems where moisture collects and a wetter microhabitat can persist. Older infected seedlings either die or snap depending on the place of the lesion. Snapped seedlings can develop a new leader or produce a new shoot from the base because the pathogen does not infect roots (Lilja and others 1996, 2007).

Because *P. cactorum* can overwinter in organic material, nursery hygiene such as removing diseased, zoospore-producing seedlings and plant debris has proven effective in the control of stem lesion. It is also important to keep the microclimate within birch seedling stands as dry and well ventilated as possible. *P. cactorum* is capable of infecting seedlings through intact bark, although disease severity was generally higher in seedlings in which inoculations were made on leaf scars (Lilja and others 1996, 2007). Thus, even small wounds can increase the risk of stem lesions. A systemic fungicide fosetyl-aluminium (800g /L) is authorized for the chemical control of this pathogen.

Birch rust

Birch rust is caused by *Melampsorium betulinum* and manifests as yellow urediniospores in late summer on the undersides of leaves of *Betula* spp. Poteri (1992) has suggested that *M. betulinum* has two *formae speciales*. When urediniospores collected from silver birch (*B. pendula*) and downy birch (*B. pubescens*) were used in cross-inoculation trials, downy birch showed partial resistance to silver birch rust in the form of necrotic lesions at infection sites and reduced production of new urediniospores. In contrast, downy birch rust was equally virulent in both birch species and no hypersensitivity reactions were found even though several different clones of silver birch were tested (Poteri 1992, Poteri and Ryyänänen 1994). Scanning electron microscopy of appressoria formation, location and penetration of stomata failed to recognize any of these factors as the basis for resistance (Poteri and Ryyänänen 1994). Silver birch clones were found to have different resistance to silver birch rust, and clones grown at low nitrogen levels were more resistant than those grown at higher levels (Poteri and Rousi 1996). Intensive culture (high seedling densities and high nitrogen supply) create wetter conditions on leaf surfaces that favor urediniospore germination (Sharp and others 1958).

M. betulinum is capable of overwintering in buds or fallen leaves as uredinial mycelium (Liro 1906) or as urediniospores (Dooley 1984). The epidemic phase of *M. betulinum* often starts in late June. It is necessary to control birch rust in nurseries because of growth reduction and high mortality after outplanting (Lilja 1973). Two mixtures are used for rust control: trifloxystrobin (187.5 g/L) and propiconazole (125 g/L), or trifloxystrobin (125 g/L) and propiconazole (125 g/L).

Grey mold

Grey mold is caused by *Botrytis cinerea* and often occurs on young seedlings when humidity is high (Sutherland and Davis 1991). Low light intensity coupled with environmental stress (e.g., 30-40 °C or prolonged drought) might also be an important predisposing factor (Zhang and Sutton 1994, Zhang and others 1995). It can also develop after damage by frost, fertilizers or herbicides (Sutherland and Davis 1991) and during seedling storage. While a short-day treatment is necessary for summer and autumn plantings and beneficial prior to freezer storage, reduced photosynthesis, increased respiration and humid conditions within the blackout curtains can weaken seedlings and encourage fungal infections, e.g., *B. cinerea*. Risks associated with high humidity and carbohydrate depletion are also high when seedlings are packed into closed boxes for storing and transportation. Venn (1981) isolated *B. cinerea* from moldy needles of bare-rooted Norway spruce seedlings in cold storage. Infections were common in the lower, shaded branches and were probably initiated in seedling beds (Venn 1979). Petäistö (2006) also found *B. cinerea* to be an important fungal pathogen during freezer storage, where an infection spread readily from diseased seedlings to healthy ones inside cardboard boxes during thawing.

Cultural pest management techniques together with chemical control are needed to avoid losses caused by grey mold. The microclimate within the canopy should be kept as dry and well aerated as possible by regulating the seedling density, irrigation and ventilation of nursery greenhouses (Mittail and others 1987). Irrigation in the morning ensures rapid drying

of foliage during the day and it has also been recommended to brush seedling tops with plastic pipe or a wooden dowel after irrigation to dislodge water droplets and encourage drying (James and others 1995). There are situations, however, when fungicide treatment of *B. cinera* is needed, e. g., packing of seedlings into tight boxes and during winter storage (Venn 1979, Petäistö 2006). A number of fungicides are used for the control of grey mold. Today three products having iprodione (750 g/L) or thiophanate-methyl (700 g/L) as their active ingredient and are authorized for use in this respect.

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BIOECOLOGY AND MANAGEMENT OF WHITE GRUB COMPLEX IN TEAK FOREST NURSERY IN INDIA

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ABSTRACT

White grubs, by virtue of their cosmopolitan distribution and feeding habits, are major pests of forest nursery crops, agricultural crops and turf grasses in India and abroad. Research on incidence and management of white grubs in a major central Indian teak (*Tectona grandis* Linn. f.) nursery (area 32 hectare.) revealed presence of three scarab species, viz., *Holotrichia rustica* (Burm.), *H. mucida* (Gyll.) and *Schizonycha ruficollis* (Fab.). While the adults (beetles) fed or mated on naturally growing bushes in the nursery, the immature stages (grubs) of these species fed on roots of teak seedlings immediately after emergence in the nursery beds. Damaged teak seedlings succumbed to the root injury leading to mortality. Prior to this research, these three white grub species were not known to damage any forestry host. Severe incidence (14-52%) of these scarabaeid grubs caused great economic losses and hampered the production of quality seedlings required for routine plantation program by the State Forest Department.

This paper discusses studies conducted in two phases. Phase I was to understand the preliminary biology in laboratory and field, viz., monitoring of adults (beetles) in relation to the meteorological factors, duration of activity, beetle and white grub density, host range in the specific area, and management related experiments that included photosensitivity of beetles. Phase II focused on a strategic integration of best feasible and economical methods for management of these scarab species within sustainable limits, under the globally accepted concept of Integrated Pest Management (IPM).

INTRODUCTION

Rich amounts of nutrition in monoculture planting stock of forest nurseries invite herbivory by many insect pests (Browne 1968). White grubs of scarabaeid beetles are the major reported pests on a variety of forest nursery stock (Beeson 1941, Mathur 1960, Browne 1968). In India, nearly 20 species of white grub out of 10 genera are known as pests on agricultural crops (Yadav and Sharma 1995, Musthak Ali 2001, Anitha and others 2006); whereas 5 species of 2 genera, *Holotrichia* and *Schizonycha*, are pests on forestry crops in nurseries (Garg and others 2005, Kulkarni and others 2006, 2007). The active species of major concern in teak (*Tectona grandis*) nurseries are *H. serrata* Fab., *H. consanguinea* Blanchard and *H. reynaudi* Blanchard (syn. *H. insularis* Brenske) (Oka and Vaishampayan 1979, Meshram and others 1993, Thakur 1993, Garg and others 2005). These species cause heavy losses in teak seedlings in India. One possible explanation for the lower number of

species known to occur on forestry crops could be improper grouping of beetles under *H. consanguinea*, which is based solely on morphology similarities (Chandra 1989).

Due to insufficient information on bioecology of white grubs in teak nurseries, management efforts have mainly depended on chemical treatment of beds for controlling grub population density in the soil. Chemical treatments are being used despite being uneconomical and having a negative impact on the environment. In view of the importance of species- and locality-specific field data on an insect pest for an effective IPM program, the author has reviewed research accomplishments during a recently reported epidemic of three scarab species (*Holotrichia rustica*, *H. mucida* and *S. ruficollis*) in a central Indian teak (*Tectona grandis* Linn. f.) nursery (Kulkarni and others 2007, 2009). Data on bioecology of the pests were reported with an aim to develop a package of practices under an IPM program.

LIFE CYCLE OF THE SCARAB SPECIES

The appearance of all three scarab species coincided with the onset of monsoon in the second or third week of June. Their exact date of emergence was compared with the meteorological data available for the region. Mating, oviposition and hatching of grubs in the nursery beds coincided with the germination of teak seeds. Physical inspections of the nursery beds revealed the grubs living near the roots of seedlings, feeding on the fine rootlets and main roots, thereby leading to external symptoms of wilting and plant mortality. Life cycles of scarab beetles are normally annual, except for a few species (Yadav and Sharma 1995). Scarab beetles (adults) emerge from the soil at night after the first heavy pre-monsoon or monsoon showers during June or July. The beetles aggregate on food plants for feeding and other hosts for mating purposes during the night before reentering the soil to lay eggs prior to the dawn. Once the beetles become sexually mature and emerge, the cycle continues daily (activity period is species and locality-specific). The newly hatched grubs inside the soil grow by feeding on the roots and rootlets, at the cost of precious planting stock material, reaching maturity by the end of October. They remain there until they develop into adults and emerge during the next monsoon (Yadav and Sharma 1995, Kulkarni and others 2007, 2009).

EMERGENCE AND ACTIVITY PERIOD

Adult Stage

Kulkarni and others (2007, 2009) have found the scarab complex of *H. rustica*, *H. mucida* and *S. ruficollis* to emerge during 2nd to 3rd week of June or July (Fig. 1 and 2). Date of emergence depends upon onset of first heavy pre-monsoon or monsoon rains in presence of rising atmospheric relative humidity (RH%) > 50%, and lowering temperature 2-3 weeks prior to heavy rains (Kulkarni 2009). This rise in RH is a characteristic of Indian climate (Raju and others 2005). In the presence of higher RH even a small amount of rain induces beetle emergence; however, rains in absence of RH did not induce emergence (Kulkarni 2009). The beetles were active in the field after dusk only for 18-19 days. During this time they emerged, mated on host food/ non-food plants every night, returned to the teak beds

before the sunrise and laid eggs. The short activity period of these three scarab beetles proved useful for developing management strategy (Kulkarni and others 2009) (Fig. 3).

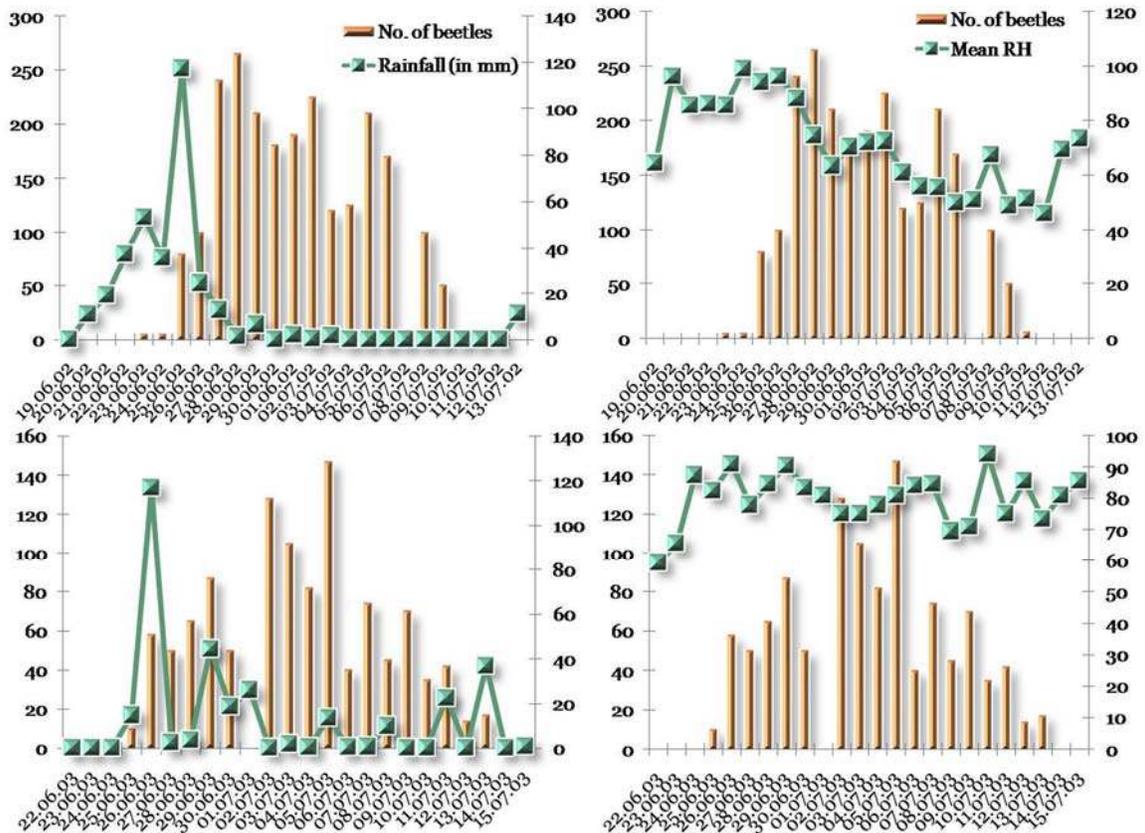


Figure 1. Day-wise emergence of beetles vis-à-vis rainfall and relative humidity in 2002 and 2003.

Activity of Indian adult scarabs is known occur with the arrival of monsoon or heavy pre-monsoon rains, but correlation with the other environmental parameters was never demonstrated prior to research by Kulkarni (2009) and Garg and others (2005). Garg and others (2005) also demonstrated strong positive correlation of teak seedling damage by *H. serrata* grubs with relative humidity and temperature in central Indian teak nursery. Such previously unknown information on these scarabs will be useful for planning IPM.

Immature Stage

Mating, oviposition and hatching of grubs in the nursery beds coincided with the germination of teak seeds. Physical inspections of the nursery beds revealed the grubs living near the roots of seedlings, feeding on the fine rootlets and main roots of seedlings, thereby leading to serious root damage with external symptoms of wilting and plant mortality. Incidence of seedling wilting in the nursery teak beds ranged from 8 to 42 m⁻² with mean incidence of 19.73±7.53 m⁻² (Kulkarni and others 2009).

Mortality of seedlings started two weeks after the first emergence of the beetles, *i.e.*, July onwards and continued until September. During the period, average plant mortality caused by

white grubs in nursery beds without treatment ranged 6.92 – 52.44 per cent, mean incidence being 26.24±16.67 percent. Kulkarni and others (2007) have shown the relationship between percentage of dead or dying seedling and grub density to be significantly positive and strong.

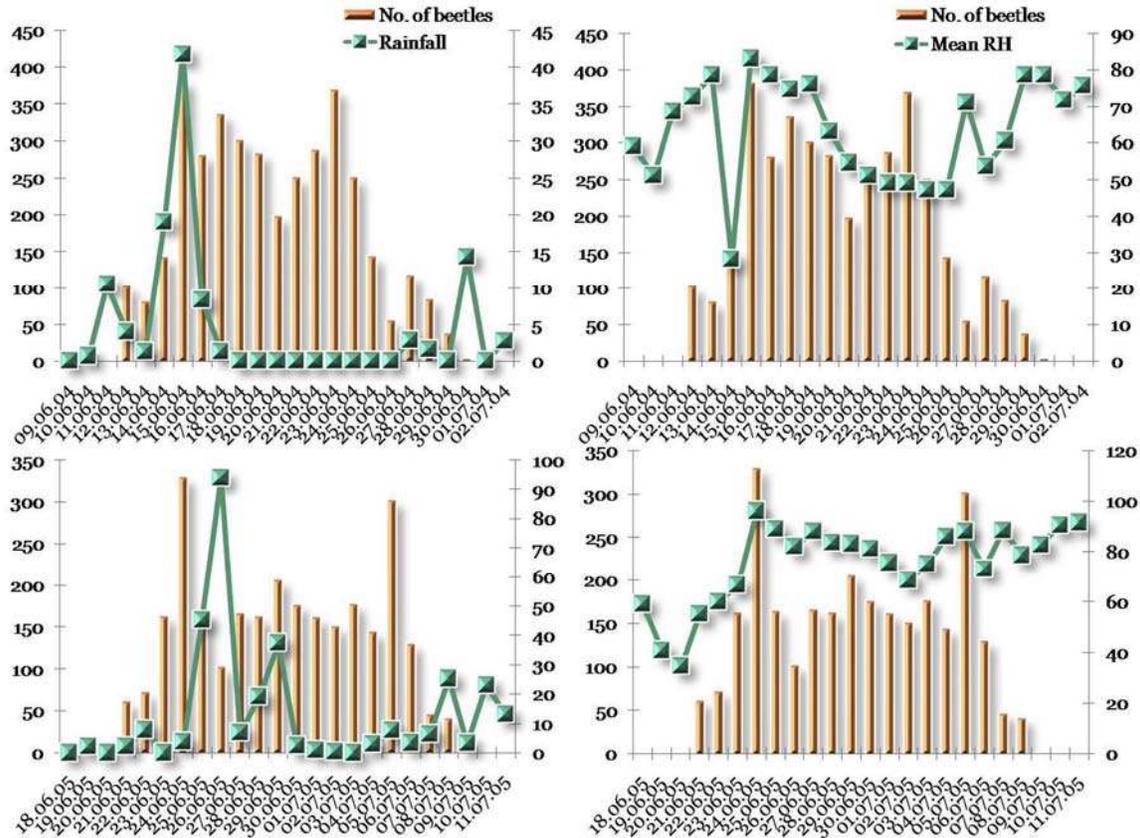


Figure 2. Day-wise emergence of beetles vis-à-vis rainfall and relative humidity in 2004 and 2005.

HOST PREFERENCES AND DENSITY

The beetles of *H. rustica*, *H. mucida* and *S. ruficollis* have common hosts. After their emergence every night, they settled on bushes of *Z. jujuba*, *Z. mauritiana*, *Acacia leucophloea*, *A. catechu* and *Z. xylopyra* growing wild on the bounds of nursery plots. However, only *Z. jujuba*, *Z. mauritiana* and *Z. xylopyra* were preferred as food plants. After their feeding they settled on other plant species for mating (Kulkarni and others 2007, 2009). Trap plants around nursery plots with a history of maximum attacked nursery beds hosted a relatively high number of beetles, indicating beetles are short fliers and search for the nearest available preferred host soon after emergence from the soil. *Ziziphus* species are preferred by a few scarab species like *H. consanguinea*, *H. serrata*, *H. rustica*, *H. mucida* and *S. ruficollis* (Beeson 1941, Oka and Vaishampayan 1979, Vaishampayan and Bhandari 1981, Kulkarni and others 2007, 2009)(Fig. 4).

Ziziphus species have been reported as food host for scarab species such as *H. consanguinea*, *H. intermedia*, *H. proplemetica*, and *H. serrata* in some parts of the country including Maharashtra (Beeson 1941, Oka and Vaishampayan 1979; Vaishampayan and Bhandari 1981, Anonymous 1985, Thakur 1993). The previous known information on host preferences by these scarab beetles were on tamarind leaves, rose leaves (Roonwal 1954), grapevine (Batra and others 1973), *Prosopis cineraria* (Parihar and Singh 1998), *Ziziphus*, tamarind and neem (Musthak Ali 2001).

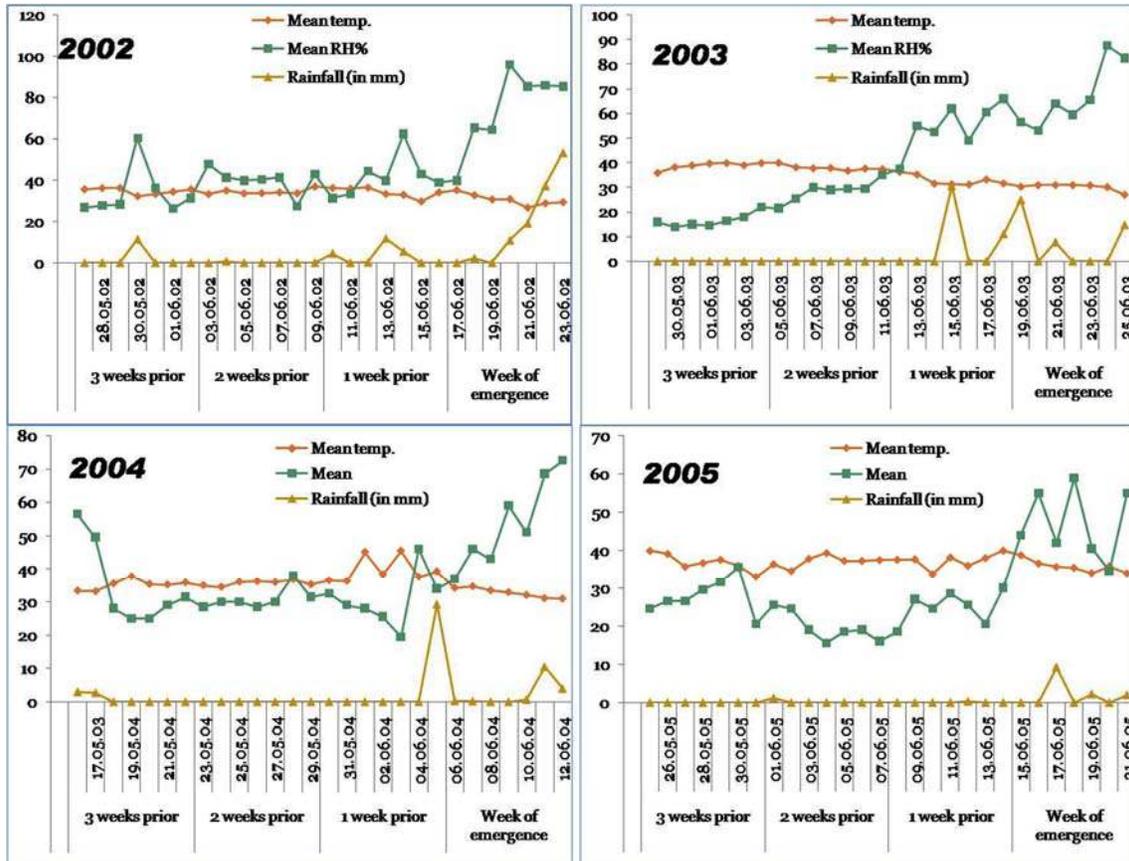


Figure 3. Trend of rainfall, relative humidity and temperature 3 week prior to the beetle emergence during 2002 to 2005.

POPULATION DENSITY OF THE BEETLES

Kulkarni and others (2007, 2009) have reported population density of *H. rustica* to be higher than *H. mucida* and *S. ruficollis*. Grubs of *H. mucida* and *H. rustica* were also observed to damage the root system of the teak seedlings, causing a wilting effect and death with larval density ranging from $6.70 \pm 2.75 \text{ m}^{-2}$ to $8.60 \pm 2.63 \text{ m}^{-2}$ in nursery beds.

OVIPOSITION BEHAVIOUR

Beetles of *H. mucida*, *H. rustica* and *S. ruficollis*, immediately after their emergence, mated and oviposited in the newly prepared beds (sown during the month of May) with loose soil. Hatching of grubs in the nursery beds coincided with the germination of teak seeds. The newly hatched immature stages, or grubs, fed on rootlets and roots of young seedlings causing mortality (Kulkarni and others 2007, 2009).

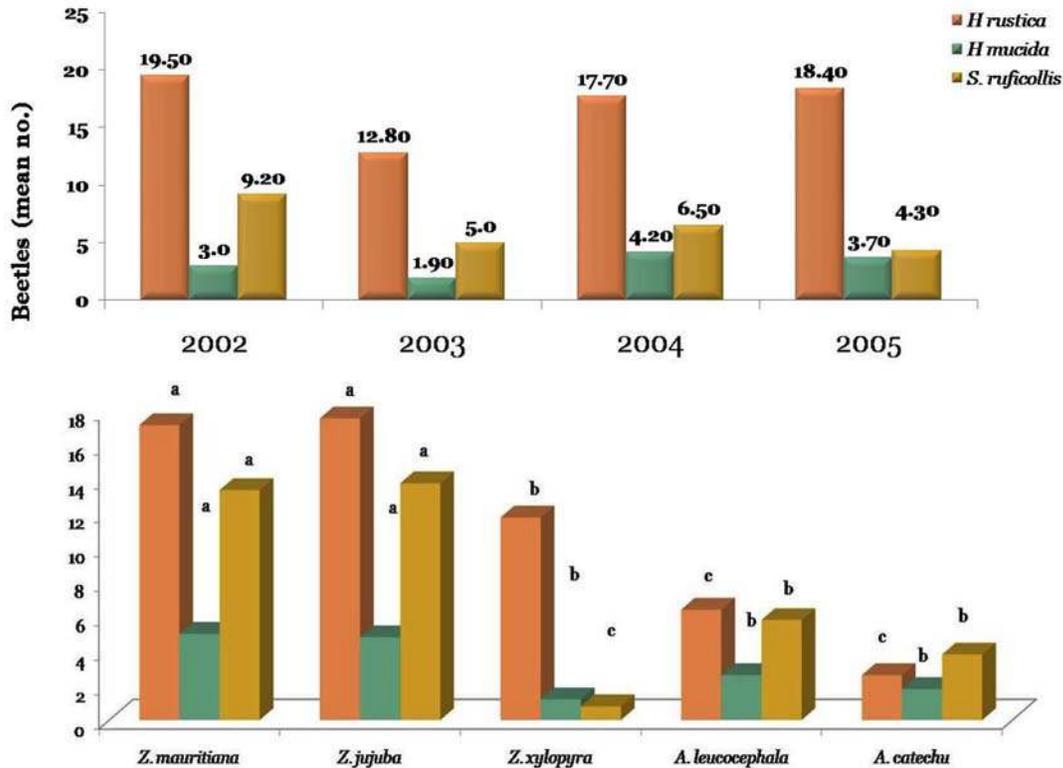


Figure 4. Beetle density and host preference.

MANAGEMENT OF THE WHITE GRUBS

Yadav and Sharma (1995) had indicated the necessity to develop an integrated program for the management of white grubs infesting agricultural crops. He had stressed to utilize the information of preferred host/s of the concerned scarab species to attract and kill the beetle and thereby to reduce the number of eggs in the soil. In practice, no detailed study was ever taken up to develop the IPM Model. Recently, Kulkarni (2009) developed time-bound guidelines as an IPM model against *H. rustica*, *H. mucida* and *S. ruficollis*. These scarab species are not photosensitive and therefore light trapping is not feasible (Kulkarni 2009). This information, in combination with the chemical treatment of nursery beds as reported by Kulkarni and others (2007) has proved effective in managing the population of the above pests below a sustainable level. Chemical control of white grubs of *H. consanguinea* (Vaishampayan and Bhandari, 1981) and *H. insularis* (Meshram and others 1993) by phorate 10G and chlorpyrifos 20 EC has been effective. Anitha and others (2006) have reported

seed dressing with chlorpyrifos and imidacloprid to be effective against *H. serrata* and *H. renaudi* in southern India on groundnut farms. For controlling white grubs of *S. ruficollis* treatment of teak nursery beds with phorate 10G at 20g/m² and chlorpyrifos 20EC at 5ml/m² can be recommended. Kulkarni and Paunikar (2009) have also recorded a possible elaterid predator, *Agrypnus fuscipes*, with potential of consuming 4 to 11 grubs per day, which can be part of a management program for the scarab species in India.

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THE USE OF PROTHIOCONAZOLE TO CONTROL FOREST NURSERY DISEASES OF *PINUS* SPP

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ABSTRACT

Laboratory, greenhouse and field trials have shown Proline[®] to be efficacious against three fungal pathogens that cause damage and seedling mortality in forest-tree nurseries. Disease control using Proline[®] has been obtained at 365 ml/ha (5 fl oz/acre) for the control of fusiform rust (*Cronartium quercuum* f.sp. *fusiforme*) on loblolly pine (*Pinus taeda*) in both greenhouse and field trials. In greenhouse trials, a biweekly application, 365 ml/ha, (5 fl oz/acre) controlled *Fusarium circinatum* (Pitch Canker) on longleaf pine (*Pinus palustris*) and resulted in an 11% increase in seedling production over non-treated seedlings. *In vitro* studies using Proline[®] amended agar media resulted in 100% fungicidal control against *Fusarium circinatum* at all 5 rates used: 0.0625x, 0.125x, 0.25x, 0.5x and 1x the recommended label rate. A biweekly application of Proline[®], 402 ml/ha (5.5 fl oz/ac), in nursery field tests significantly reduced *Rhizoctonia* foliar blight on loblolly pine when compared to applications of azoxystrobin and the non-treated control. The monetary loss per hectare due to *Rhizoctonia* foliage blight was \$10,864, \$4,198 and \$44 for non-treated, azoxystrobin and Proline[®] respectively. In addition to disease control, Proline[®] treated seedlings were significantly larger and appeared greener than non-treated seedlings.

INTRODUCTION

The availability of fungicides to control specific forest seedling nursery diseases is either nonexistent, limited or faces possible loss of US label registration. Of the many insects and diseases that occur in forest-tree nurseries, three fungal pathogens stand out as problematic in southern US nurseries. These diseases include Fusiform Rust, Pitch Canker, and *Rhizoctonia* Foliar Blight. The most important disease of loblolly (*Pinus taeda*) and slash (*Pinus elliotti*) pine seedlings is fusiform rust caused by *Cronartium quercuum* f.sp. *fusiforme*. Since 1980, formulations of Bayleton[®] (triadimefon) have been the primary chemical used to control this disease (Carey and Kelley 1993) and has consistently provided excellent cost-effective control as both a seed treatment and foliar spray (Snow and others 1979, Carey and Kelley 1993, Carey 2004).

In July 2007, Bayer CropScience received US Environmental Protection Agency's (EPA) cancellation order for Bayleton[®]. While most of the food and non-food crops such as apples, pears, grapes and raspberries were removed from the US label, its use on pine seed and seedlings was still allowed. However, the availability and formulation remain unsettled, resulting in nurseries having difficulty locating and obtaining the product; an alternative is needed.

Pitch canker, caused by the fungus *Fusarium circinatum*, can cause significant seed and seedling mortality in nurseries and later after outplanting in the field (Carey and Kelley 1994, Dwinell 1978, Barrows-Broadus and Dwinell 1984, Blakeslee and Rockwood 1984, Lowerts and others 1985, Kelley and Williams 1982, Dwinell and Barrows-Broadus 1981). In the southern US, infection and seedling losses have been reported on loblolly, slash, longleaf (*Pinus palustris*), shortleaf (*Pinus echinata*) and Virginia (*Pinus virginiana*) pine. The fungus is also considered one of the most threatening diseases in many areas of the world, particularly the South African nurseries (Storer and others 1998, Viljoen and Wingfield 1994). Unlike fusiform rust, there are no fungicides registered for the control of pitch canker on either seed or seedlings and nursery growers are forced to use either bleach or hydrogen peroxide to disinfect seed.

Longleaf and loblolly pines are particularly susceptible to *Rhizoctonia* foliar blight. The disease is caused by a species of *Rhizoctonia* spp. or binculeate forms of sexual states belonging to the genera *Thanatephorus* or *Ceratobasidium*. *Rhizoctonia* foliar blight can cause significant pine mortality in nursery beds and typically occurs in late July when the seedling canopy closes in (Carey and McQuage, 2003). Symptoms of dead and dying needles and seedling mortality appear in patches within the bed where moisture and temperature favor infection. Many times the disease is not observed until seedlings are top-clipped to maintain seedling shoot:root ratios and heights. Varying degrees of resistance among seedling families can be found, with US gulf coastal seedlots more susceptible than Piedmont sources, and the disease is rarely observed on slash pine (McQuage, 2009 personal communication). *Rhizoctonia* foliar blight is not distributed uniformly throughout a nursery and is generally limited to isolated foci and the disease is also more severe in second crop fields. While there are fungicides registered for *Rhizoctonia* foliar blight, they are not always efficacious (Carey and McQuage 2004).

In an attempt to find an alternative for the control of fusiform rust, trials examining numerous fungicides by have been underway since 2004. In 2008, Proline[®] 480 SC (41% prothioconazole, Bayer CropScience) was examined as it had a broad spectrum systemic control of ascomycetes, basidiomycetes, and deuteromycetes on numerous field crops. Prothioconazole belongs to the new chemical class of triazolinthiones (Mauler-Machnik and others 2002) and inhibits the demethylation process at position 14 of lanosterol or 24-methylene dihydrolanosterol, which are precursors of sterols in fungi. Prothioconazole efficiently stops many steps of the fungal infection chain like appressoria and haustoria formation, mycelial growth as well as spore formation. Currently Proline[®] is registered in the US for food crops including peanuts, barley, wheat, sugar beets and soybeans.

Although Proline[®] is not currently registered for commercial use in US forest-tree nurseries, these studies examined Proline[®] in laboratory, greenhouse and field trials to determine if the fungicide was efficacious against the three fungal pathogens that are capable of causing significant damage and seedling mortality in forest-tree nurseries. Data collected from such studies will be used in an attempt at obtaining a full-use label from Bayer CropScience and US EPA for disease control in forest-tree nurseries in the southern US.

METHODS

Fusiform Rust Greenhouse Trials

Seed treatments. In 2006, 2007 and 2008 loblolly pine seed were stratified for 4 weeks, after which they were treated with fungicides prior to sowing (Table 1). For dry formulation fungicides, seed was first moistened in a seed tumbler, and the fungicide was added at the rate of 25 g/10 kg (2 oz/50 lbs) of seed. For liquid fungicides approximately 26 ml (2 fl oz) of the product was used per 10 kg (50 lbs) seed which was slowly added to pine seed in a tumbler. The fungicide and seed was tumbled until dry. All treated seed, as well as non-treated seed for both positive and negative controls, were double sown to Ray-Leach[®] containers and then thinned to one seedling per cell as they germinated.

Table 1. Fungicide rates, actual product per unit, used in 2006, 2007 and 2008.

Treatments	Active Ingredient	Foliar Treatment ¹		Seed Treatment
		1X	2X	1X
Check (water)				
Bayleton [®]	tridimefon 50%	560 ml/ha (8 oz/a)		25 g/10 kg seed (2 oz /50 lb seed)
Folicur [®]	tebuconazole 38.7%	292 ml/ha (4 fl oz/a)	584 ml/ha (8 fl oz/a)	
Provost [®] 433 SC	prothioconazole 12.9% tebuconazole 25.8%	621 ml/ha (8.5 fl oz/a)	1.24 l/ha (17 fl oz/a)	25 g/10 kg (2 fl oz 50 lb)
Proline [®] 480	prothioconazole 41%	365 ml/ha (5 fl oz/a)		25 g/10 kg (2 fl oz 50 lb)

¹ Based upon 280 l/ha (30 gal of water/acre)

Foliar treatments. Loblolly pine seed were stratified for 40 days and then double sown to Ray-Leach[®] containers. Following germination, containers were thinned to one seedling per container and then randomly assigned fungicidal treatments. Seven weeks post-sowing, seedlings were treated at the Auburn University's Pesticide Research Facility. A Bayleton[®] and a water check were included for both positive and negative controls, respectively. Application rates for each fungicide included a 1x and 2x rate (except Bayleton[®] which only had a 1x rate) as listed in Table 1. Proline[®] was only tested in 2008 at the 1x rate. After spraying, seedlings were returned to the greenhouse to dry.

Inoculations. One day following the foliar fungicide application, the seedlings were transported to the USDA Rust Screening Laboratory in Asheville, North Carolina. Seedlings were allowed to acclimate to the new growing conditions for 5-7 days and then challenged with 20,000 basidiospores/ml of *Cronartium quercum f.sp. fusiforme* (collected from Zone 7 inoculum area) using the laboratory's standard inoculation protocols. Seedlings remained under the care of the USDA Rust Laboratory for the duration of the growing season. At 3 and 6 months post-inoculation, seedlings were evaluated for swellings along the main stem, an indication of basidiospore infection.

Fusiform Rust Field Trials

In 2008, two nurseries (South Carolina Forestry Commission Nursery in Trenton, SC and Arborgen Nursery in Shellman, GA) participated in testing Proline[®] operationally on several nursery blocks. Proline[®], Provost[®] and Bayleton[®] were compared to a non-treated control. At each nursery a randomized complete block design was used with treatments replicated 3 times at one nursery (SC) and 5 times at the other (GA); 0.24 ha (0.6 acre) and 0.405 ha (1.0 acre), respectively. Each replication/treatment was applied to either 3 adjacent nursery beds or a 9-bed nursery section using standard nursery spray equipment. Proline[®] and Provost[®] were applied 365 ml/ha (5 fl oz/acre) and 621 ml/ha (8.5 fl oz/acre), respectively, as well as the standard Bayleton[®] application. At the end of the growing season (December 2008), seedlings were collected from each treatment plot and examined for rust infection and measured for seedling quality. In addition, seedlings were collected from the nursery in February 2009 and outplanted at a site near Auburn, AL to monitor for any long-term effects of the fungicide treatments on seedling survival.

Pitch Canker Laboratory Trials

Laboratory fungal growth studies were conducted to determine if *Fusarium circinatum* was able to grow on agar media amended with varying concentrations of Proline[®] and Pageant[®] - BASF (Table 5). Potato Dextrose Agar (Difco[®] PDA) was amended with each fungicide after autoclaving and just before pouring the plates. Twenty plates of each fungicide concentration and 20 non-amended PDA plates as a control were used. A #4 cork-borer (8 mm) plug of *Fusarium circinatum*, taken from a 2-wk-old culture, was placed at the center of each plate. The radial growth of the fungus was measured over a period of 11 days. To determine if the treatments were either fungicidal (killed the fungus) or fungistatic (stopped fungal growth), 11 days after placing onto the amended media, the agar plugs within each treatment were removed and plated onto non-amended media. Fungal growth on the non-amended media was recorded for another 5 days.

Pitch Canker Greenhouse Trials

Longleaf seed known to be infested with *Fusarium circinatum* was stratified for 10 days and sown to Ray Leach[®] containers in the greenhouse in May 2008. To ensure disease and increase fungal pressure, an 8 mm agar plug from a 2-wk-old stock culture of *Fusarium circinatum* was added to ½ of the container cavities at the time of sowing. After sowing longleaf seed, all cavities were covered with a thin layer of coarse perlite and misted. In addition to the fungal plug of *Fusarium circinatum*, ½ of the containers were sprayed with Proline[®] at sowing and every 2 weeks throughout the study. There were 20 container sets sown to longleaf pine, each container set had 20 cavities for each treatment as follows: Trmt #1 = *F. circinatum* & no Proline[®] spray, Trmt #2 = *F. circinatum* & Proline[®] spray, Trmt #3 = No *F. circinatum* & no Proline[®] spray, Trmt #4 = No *F. circinatum* & Proline[®] spray. Following germination, seedling counts were measured weekly for 4 weeks and then once per month until October 2008. Samples of dead seedlings were later assayed to confirm the presence of *Fusarium circinatum*.

Rhizoctonia Foliar Blight Laboratory Trials

Laboratory fungal growth studies were conducted to determine if *Rhizoctonia solani* was able to grow on agar media amended with Proline[®] at 1x, 0.25x and 0.0625x the label rate of 365 ml/ha (5 fl oz/ac). Potato Dextrose Agar (Difco[®] PDA) was amended with Proline[®] after autoclaving and just prior to pouring the plates. There were 20 PDA plates of each fungicide concentration and 20 non-amended PDA plates used as a control. A #4 cork-borer (8 mm) plug of *Rhizoctonia solani* taken from a 12-day old culture was placed at the center of each plate. The radial fungal growth was measured over a period of 7 days. To determine if the treatments were fungicidal (killed the fungus) or fungistatic (stopped fungal growth), 7 days after placing the plugs onto the media, the agar plugs were removed from the amended agar media and placed onto a non-amended agar plate. Fungal growth on the non-amended agar plate was recorded for another 5 days.

Rhizoctonia Foliar Blight Field Trials

In 2008 a nursery in Mississippi tested Proline[®], 402 ml/ha (5.5 fl oz/ac), and Heritage[®] (50% azoxystrobin – 1.68 kg/ha (24 oz/acre)) operationally for the control of *Rhizoctonia foliar blight*. A randomized block design with four replications was used in a nursery section growing its second seedling crop following soil fumigation. Each replication plot was 12 m x 18 m wide with a non-treated plot (6 m x 18 m) left in the middle of the field as the disease control. Fungicides were applied on a two week interval beginning July 15, 2008 using a Hardee 1532 liter sprayer with a 9-bed spray boom with nozzles on 0.5 m centers. A total of 8 applications of both fungicides were made. Temperature and relative humidity 25.4 cm above the seed bed were recorded using a HOBO[®] data logger.

In early December 2008, seedling densities, disease incidence, severity and seedling loss were calculated in 2 subplots within each treatment plot. From each subplot, 30 seedlings were hand-lifted and later measured to determine seedling quality, root collar diameter, height, dry weight and root morphology for each treatment.

RESULTS

Fusiform Rust Greenhouse Trials

The Southern Forest Nursery Management Cooperative has tested many fungicides over the years looking for an efficacious alternative for Bayleton[®] (Carey 2004, Starkey and Enebak 2008). The first fungicide tested that provided disease control equal to or better than Bayleton[®] was Provost[®] (Fig. 1). Provost[®] is made up of prothioconazole and tebuconazole (Table 1) however, when Folicur[®] (tebuconazole) was tested, 50% of the seedlings formed fusiform rust galls and it was determined that disease control achieved with Provost[®] was due to the prothioconazole portion within that compound. When it came time to re-examine Provost[®], a technical representative suggested testing a new fungicide, Proline[®] (prothioconazole) which was first registered in the US in 2007. In subsequent greenhouse trials, Proline[®] provided control of fusiform rust on loblolly pine equal to or greater than Bayleton[®] as a foliar spray (Fig. 1, Table 3). In addition, when tested as a seed treatment, there was no reduction in seed germination and Proline[®] had disease control equal to that obtained with the current standard Bayleton[®] (Table 2).

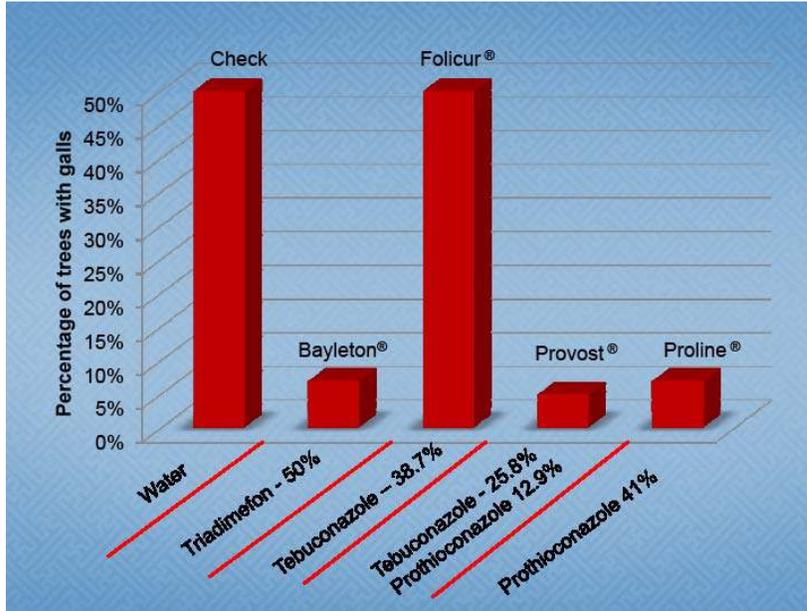


Figure 1. Three year average fusiform rust control on loblolly pine using foliar applications of fungicides.

Table 2. Seed treatment rates, germination and mean percent fusiform rust infection – 2008.

Seed Treatment Fungicides	% Germination	% Infection
Bayleton®	92%	0.0% a
Provost® 433 SC	96%	0.0% a
Proline® 480 SC	96%	1.0% a
USFS Check Seedlings		45%

Table 3. Foliar treatment rates and mean percent fusiform rust infection – 2008.

Foliar Treatment Fungicides	Foliar Rate ¹	% Infection
Bayleton®	560 g/ha (8 oz/a)	7.1% a
Provost® 433 SC	621 ml/ha (8.5 fl oz/a)	2.5% a
Proline® 480 SC	365 ml/ha (5 fl oz/a)	6.9% a
USFS Check Seedlings		45%

¹ Based upon 280 l/ha (30 gal of water/acre)

Fusiform Rust Field Trials

At the South Carolina Forestry Commission's Nursery in Trenton, SC, the level of rust infection in the control plots was zero so the ability of Proline[®] to control fusiform rust infection in the field at that location could not be properly evaluated. However, at the Arborgen Nursery in Shellman, GA, 54% of the seedlings in the control plots were infected and had developed the characteristic symptom of stem swellings and galls by the end of the growing season in December 2008. No stem swellings or galls were detected on seedlings in any of the Proline[®], Provost[®] or Bayleton[®] treated plots. There were no differences in the seedling quality (RCD, biomass) among the treatments except for seedling height in the control plots. Seedlings in the control plots were significantly taller than the three fungicidal treatment plots; this was most likely due to the seedlings in the control plots not getting top-clipped at the end of the season (the nursery was not going to sell the non-treated seedlings). Proline[®]-treated seedlings had significantly longer roots and a larger number of root tips than seedlings in the non-sprayed control plots (Table 4). Six months after outplanting, Proline[®]-treated and non-treated seedlings were similar in height and survival.

Table 4. Root length, average root diameter, root volume and number of root tips for each fungicide treatment¹.

	Total Root Length (cm)	Average Root Diameter (mm)	Root Volume (cm ³)	# of Root Tips
Proline [®]	320.7 a	0.59 a	0.89 a	854.1 a
Provost [®]	304.3 a	0.61 a	0.88 a	827.3 a
Bayleton [®]	287.8 ab	0.60 a	0.82 a	798.1 a
Control	241.4 b	0.63 a	0.76 a	683.6 b
lsd.05	53.4	0.04	0.21	105

¹Within column means followed by same letter do not differ at 0.05 level using Duncan's Multiple range Test

Table 5. Fungicide, active ingredient and rate used in *Fusarium circinatum* amended media trial.

Fungicide	Active Ingredient	Rate	ppm
Proline [®] 480 SC	prothioconazole – 41%	1x = 365 ml/ha (5 fl oz/a) ¹	1300
		0.5x = 183 ml/ha (2.5 fl oz/a)	650
		0.25x = 91 ml/ha (1.25 fl oz/a)	325
		0.125x = 46 ml/ha (0.625 fl oz/a)	162
		0.0625x = 23 ml/ha (0.321 fl oz/a)	81
Pageant [®]	pyraclostrobin 12.8% boscalid 25.2%	1x = 104.8 g/100 l (14 oz/100 gal)	1100
		0.5x = 52.4 g/100 l (7 oz/100 gal)	550
		0.25x = 26.2 g/100 l (3.5 oz/100 gal)	225

¹ Based upon 280 l/ha (30 gal of water/acre)

Pitch Canker Laboratory Trials

In vitro fungal growth on agar media amended with Proline[®] resulted in 100% fungicidal control against *Fusarium circinatum* as fungal growth did not occur on any of the Proline[®]-amended PDA plates for any concentration examined for the 11-day experiment (Fig. 2). All

six rates of Proline[®] are at 0 mm on the Y-axis in Figure 2. On some Proline[®]-amended plates, the fungus grew from the original 8 mm plug for several mm, but never touched the agar surface. The appearance was that of a mushroom cap suspended over the soil. *Fusarium circinatum*, while somewhat inhibited on Pageant[®]-amended agar, was able to grow on all concentrations of Pageant[®] tested. There were no differences among the 3 concentrations of Pageant[®] tested. *Fusarium circinatum* growth on the non-amended control plates was significantly greater than either Pageant[®]- or Proline[®]-amended plates.

After 11 days, the plugs were removed from the amended media and put onto non-amended agar media. None of the agar plugs from the Proline[®] amended plates resumed fungal growth when returned to non-amended agar indicating that Proline[®] was fungicidal to *Fusarium circinatum*. However, agar plugs from the Pageant[®] amended media did resume growth on the non-amended agar indicating that Pageant[®] was fungistatic to *F. circinatum*.

Pitch Canker Greenhouse Trials

A biweekly application at 365 ml/ha (5 fl oz/a) on longleaf pine to control pitch canker (*Fusarium circinatum*) resulted in an 11% increase in seedling production over non-treated seedlings with no fungal plug added (Table 6). The percentage of seedlings obtained for no fungal plug and no Proline[®] (Trmt #3) is what a nursery sowing this seed would expect to obtain without fungicidal control. The same relationship held true with cavities that had a fungal plug added (increased disease pressure), for example, cavities with *F. circinatum* added to the cavity and no Proline[®] sprays had 62% fill at week 11. This was significantly less than cavities with no fungal plug and Proline[®]. Cavities with a fungal plug and Proline[®] had 17% greater fill percentage than without Proline[®]. Dead seedlings from Trmt #2 and #4 tested positive for *Fusarium circinatum*. Longleaf seedlings receiving Proline[®] sprays were significantly larger (height, root collar diameter and shoot dry weight) than non-Proline[®] treated seedlings.

Rhizoctonia Blight Laboratory trials

Agar media amended with Proline[®] resulted in 100% control against *Rhizoctonia solani* as fungal growth did not occur on any of the Proline[®]-amended PDA plates for any concentration used for the 7 day experiment (Fig. 3). All three rates of Proline[®] are at 0 mm on the Y-axis in Figure 3. After 7 days, the plugs were removed from the amended media and placed onto non-amended agar media. The agar plugs from each rate of the Proline[®] amended media resumed growth on the non-amended agar indicating that Proline[®] was fungistatic to *Rhizoctonia solani*.

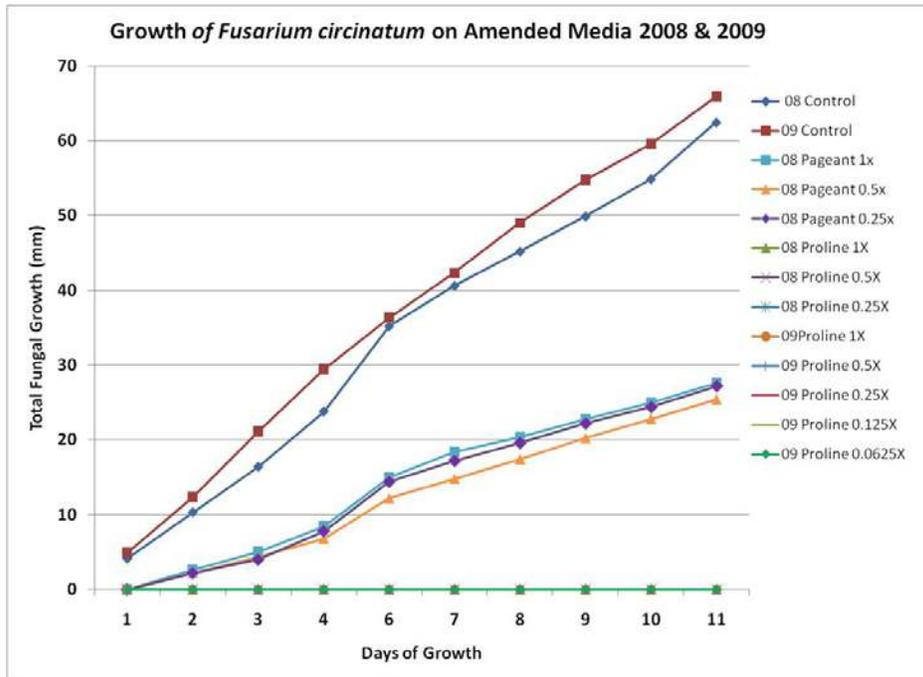


Figure 2. Radial growth of *Fusarium circinatum* on fungicide-amended and non-amended agar.

Table 6. Fill percentage and longleaf seedling quality in greenhouse pitch canker study¹.

Trmt #	Treatments	Proportion of Cavities Filled	Height (cm)	RCD (mm)	Top Dry Weight (g)
1	Fungal Plug + Proline [®]	0.79 a	32.0 a	4.6 a	1.40 a
2	Fungal Plug No Proline [®]	0.62 c	28.2 b	4.7 a	1.23 b
3	No Fungal Plug + Proline [®]	0.80 a	31.8 a	4.7 a	1.42 a
4	No Fungal Plug No Proline [®]	0.69 b	28.9 b	4.3 b	1.22 b
	lsd	0.07	0.5	0.2	0.11

¹Within column means followed by same letter do not differ at 0.05 level using Duncan's Multiple Range Test

Rhizoctonia Blight Field Trials

Disease incidence, severity and number of seedlings lost in the Proline[®]-treated plots was significantly lower than in the Heritage[®] and non-treated control plots (Table 7). An estimate of the potential loss (assuming similar incidence and severity throughout the acre area) indicated that losses from Proline[®] were negligible (0.03%). There were no significant differences in either seedling quality or root morphology, although the controls had numerically fewer seedlings (Table 7). The potential monetary loss in Table 7 reflects the seedling loss in the test plot, not the whole nursery as Rhizoctonia foliage blight tends to occur in isolated foci in susceptible seedlots. This particular nursery reported that within these susceptible seedlots, total loss to the disease would be less than 0.5%. Proline[®] was effective in reducing seedling loss due to Rhizoctonia that normally would occur. In years when the environmental parameters do not favor spread of the fungus through the seedling beds, Heritage[®] may provide a suitable level of control.

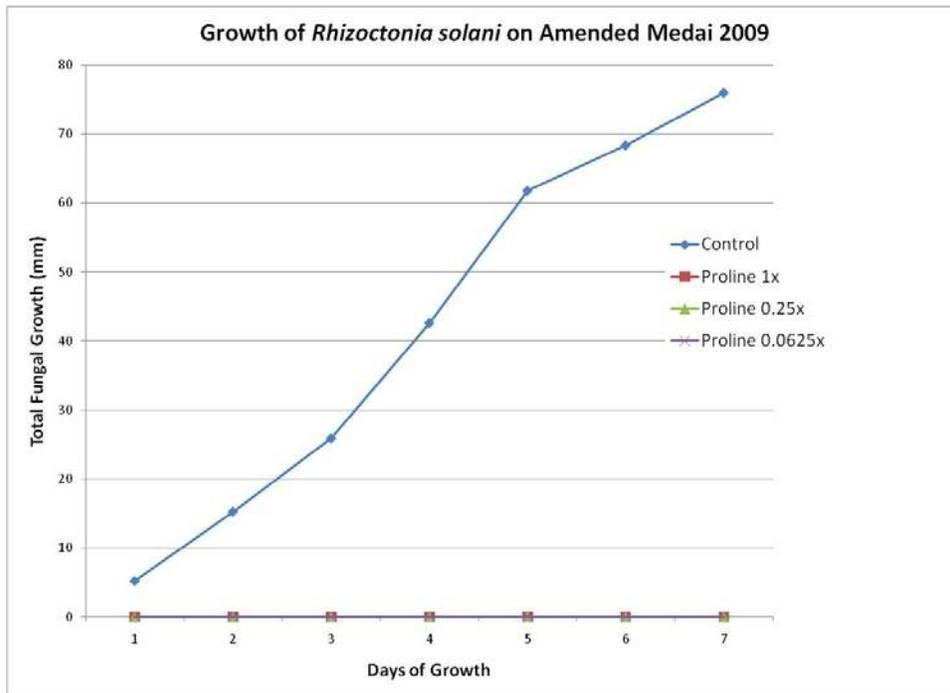


Figure 3. Radial growth of *Rhizoctonia solani* on fungicide-amended and non-amended media.

Table 7. Seedling density and disease loss as measured by incidence, severity and seedling loss per m² and potential loss per hectare caused by *Rhizoctonia* foliage blight.

Trmt	Seedling Density per m ²	Disease Incidence ¹	Disease Severity ²	Seedling loss per m ² ³	Potential Loss per ha
Control	246	0.354	0.182	32	\$10,864
Heritage [®]	254	0.162	0.083	13	\$4,198
Proline [®]	255	0.003	0.001	0.1	\$44
<i>Prob > F</i>	0.7762	0.0004	0.0004	0.0031	-

¹ Incidence = proportion of bed feet within a 1x4' frame with *Rhizoctonia* Foliar Blight

² Severity = proportion of tissue affected by *Rhizoctonia* Foliar Blight

³ Seedlings loss = # trees/drill x incidence/drill x severity /drill x seedling density

⁴ Controls were not included in the statistical analysis due to lack of replication among blocks.

DISCUSSION

Laboratory, greenhouse and field trials have shown Proline[®] to be efficacious against three fungal pathogens that cause damage and seedling mortality in forest-tree nurseries. Disease control of all three fungi using Proline[®] was obtained using rate of 365 ml/ha (5 fl oz/a) which is within the current Proline[®] range of 183 to 416 ml/ha (2.5 – 5.7 fl oz/ac) for registered crops. There is also an annual maximum use rate for each crop and these

laboratory studies show that Proline[®] is capable of controlling fungi *in vitro* at rates much lower than 365 ml/ha (5 fl oz/a). The key to any fungicide application is to apply the minimum rate necessary to control the disease and caution should be used when applying laboratory results to field or greenhouse studies. Small trials testing this product under the different environmental conditions that occur in nurseries are warranted prior to becoming operational.

Proline[®] is fungicidal to *Fusarium circinatum*, but is fungistatic on *Rhizoctonia* spp. Therefore, the timing of consecutive applications of Proline[®] would be important for the efficacious control of *Rhizoctonia* foliar blight in nurseries. Preliminary studies have shown that seed germination is not inhibited in loblolly, longleaf, slash or shortleaf pine. However, the minimum rate and method of application still must be examined as well as the minimum number of applications necessary to control pitch canker. Pitch canker losses occur either from external seed borne fungi or later in the season from seed infected internally and there may be a difference in seed treatment or foliar applications to control both of these modes of infection.

As part of the Southern Forest Nursery Management Cooperative's mission to bring new chemistry to its members, in early 2009, as a result of various experiments over the past three years, and in cooperation with Bayer CropScience, an application was filed with the US EPA in 6 southern US states for a Proline[®] 24(c) label. The intended Proline[®] label was for the control of pitch canker and *Rhizoctonia* foliar blight in loblolly and longleaf pine. Approval had been received in 5 of the 6 states when in March, 2009, US EPA requested Bayer CropScience pull the approved 24(c) labels. The US EPA determined that the forest nursery use is a new non-food use that requires a separate ecological risk assessment, and the existing data on file only supports food crops. In response to US EPA's denial, Bayer CropScience provided a response to support the continued use under the Section 24(c). In their response, Bayer's support of the 24(c) was based on several reasons including; 1) the minor acreage involved, 2) the use pattern is only for nursery and not forestry, 3) the proposed use pattern has a similar application method and exposure as the already registered crop use, and 4) the proposed use pattern poses no greater risk (or lower risk) compared to the currently registered uses. However, in the end, the US EPA did not change their ruling and Proline[®] is not yet available for forest-tree nurseries. Several other labeling efforts (IR4 and Section 18) were explored but found not feasible. The Southern Forest Nursery Management Cooperative is now pursuing requirements with US EPA and Bayer CropScience for a full product registration.

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PYTHIUM SPECIES ASSOCIATED WITH FOREST TREE NURSERIES OF OREGON AND WASHINGTON

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ABSTRACT

Pythium species are one of several pathogen genera responsible for damping-off of conifer seedlings in forest tree nurseries. Field trials were established in 2008 at three nurseries (2 in OR, 1 in WA) to: 1) evaluate the impact of lower application rates of fumigants on *Pythium* soil populations; and 2) identify *Pythium* species associated with damping-off. Six fumigant treatments (including a conventional methyl bromide treatment and a nonfumigated control) were applied according to a randomized complete block design with four blocks at each nursery. Soil samples were collected before and after fumigation and *Pythium* populations were assessed by baiting with rhododendron leaves and Douglas-fir needles and by dilution plating onto PARP, a semiselective medium for pythiaceus species. Isolates were identified based on their ITS sequence. Prior to fumigation, populations averaged 40-45 cfu/g soil at nurseries A and B and 19 cfu/g soil at nursery C. All fumigant treatments reduced soil populations by at least 86% and populations were similar 7 months after fumigation. Of the 450 isolates identified to date, 42% are *P. irregulare*, 27% are *P. dissotocum*, 10% are *P. macrosporium*, and the remaining 21% are composed of 12 different *Pythium* species.

INTRODUCTION

Forest tree nurseries of the Pacific Northwest (Idaho, Oregon, and Washington) produce approximately 200 million conifer seedlings annually. Most of the conifer seedlings grown and sold are two-year-old Douglas-fir transplants (*Pseudotsuga menziesii*). In the absence of fumigation, production can be severely limited by several root pathogens, including *Pythium* species, which cause damping-off and root rot. These pathogens infect at or below the soil line and kill young, succulent tissues of seedlings.

Traditionally, management of soilborne pathogens has been accomplished by fumigation with 392 kg/ha of methyl bromide/chloropicrin 67:33 under a critical use exemption permit. However, methyl bromide use will eventually cease as stocks become depleted under the Montreal Protocol and fumigants are becoming increasingly regulated due to safety concerns. A field study was conducted to compare disease control efficacy of lower rates of alternative fumigants to the traditional application of methyl bromide. The objectives of this portion of the study are to assess: 1) the impact of lower rates of fumigants on *Pythium* soil populations; and 2) identify the *Pythium* species associated with damping-off of Douglas-fir seedlings.

METHODS

Field trials were established at two forest nurseries in Oregon and one forest nursery in Washington. Six fumigant treatments (including a conventional methyl bromide/chloropicrin treatment and a nonfumigated control) were applied in a randomized complete block design with four replicate blocks at each nursery in early August 2008 (Table 1). Each treatment plot was approximately 12 × 46 m (nonfumigated control plots 12 × 30 m). Soil samples were collected before (1 week) and after fumigation (1 and 7 months) by taking 20 soil cores in a randomized pattern to a depth of 30 cm from each treatment plot within a block. Soil samples were bulked within each treatment by block and nursery to create composite samples.

Table 1. Fumigant treatments at each of three forest tree nurseries.

Fumigant	Application Rate	Plastic Film Type
Methyl Bromide/Chloropicrin	392 kg/ha(67:33)	High Density Polyethylene
Methyl Iodide/Chloropicrin	274 kg/ha (50:50)	High Density Polyethylene
Methyl Iodide/Chloropicrin	274 kg/ha (50:50)	Virtually Impermeable Film
Metam Sodium/Chloropicrin	467 l/ha + 137 kg/ha	Virtually Impermeable Film
Dimethyl Disulfide/Chloropicrin	561 l/ha + 135 kg/ha	Virtually Impermeable Film
Untreated	none	none

To sample for *Pythium* species, ten grams from each composite sample were added to 90 ml of 0.2% water agar and shaken for 45 minutes at 1500 rpm. Aliquots of the suspension (0.5 ml) were then spread on 10 petri plates containing PARP, a semiselective medium for Pythiaceus species (Erwin and Ribiero 1996). Plates were incubated at room temperature for two days and the number of *Pythium* isolates per plate was counted. The assay was conducted twice (two trials) for each of the three time periods of soil collection (1 week before and 1 and 7 months after fumigation).

Pythium species were also assayed using the double-cup leaf disk baiting method from Linderman and Zeitoun (1977). Briefly, 15 ml of each composite soil sample were placed in a 150-ml wax paper cup. A second wax paper cup with its bottom replaced by a double layer of cheesecloth was positioned firmly on top of the sample and 50 ml of distilled water were added. Leaves of *Rhododendron* ‘Nova Zembla’ and needles of Douglas-fir were then used to bait for *Pythium* species. Leaves and needles were initially rinsed in running tap water for 10 minutes and then surface disinfested by immersing in 0.06% sodium hypochlorite for 10 minutes. After air drying, 10 5-mm-diameter *Rhododendron* leaf disks or 10 split Douglas-fir needles were floated on the water surface in each cup at room temperature. After 48 hours, disks and split needles were removed from the cups with sterile forceps, blotted dry on clean paper towels, and plated on PARP. Plates were incubated at room temperature for two days and the number of *Pythium* isolates per plate was counted. The assay was conducted twice (two trials) for each of the three time periods of soil collection.

A subset of isolates (up to three isolates per composite soil sample, when available) was identified on the basis of the internal transcribed spacer (ITS) region. Genomic DNA was extracted by using a procedure modified from Martin and Semer (1997). Briefly, cultures of each *Pythium* isolate were grown on 20 ml of 10% clarified V8 juice agar for 3 days (1 g CaCO₃ per 100 ml V8 juice strained through eight layers of cheesecloth. Mix 100 ml clarified V8 juice in 900 ml distilled water and 17 g agar). A small amount of hyphae (< 1

mm³) was then removed from each culture with a sterile toothpick and incubated at 95.9 C for 5 minutes. Ten microliters of the DNA extract was then added to a 40 µl PCR reaction mixture containing 20 µl 2.5x 5 Prime HotMasterMix (5 Prime Inc., Gaithersburg, MD, USA), 18 µl sterile water, and 1 µl each of 10mM primers ITS1 and ITS4 (White and others 1991). Amplification was performed in a Veriti Thermal Cycler (Applied Biosystems Inc, Foster City, CA, USA) with the following temperature profile: one cycle of 1 minute at 95°C; 35 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C; and 10 min at 72°C. PCR products were separated by electrophoresis on a 0.7% agarose gel in 1x TAE buffer. Gels were stained with ethidium bromide and photographed under UV light.

RESULTS

Average prefumigation counts by dilution plating of *Pythium* for each nursery (all results reported are from trial 1 only) were greatest at nursery B (45 cfu/g dry soil) and A (40 cfu/g dry soil), and least at nursery C (19 cfu/g dry soil). Dilution plate counts of *Pythium* in all fumigant treated plots were reduced by at least 86% one month after fumigation, or by at least 95% seven months after fumigation (Fig. 1). Analyses of variance on the counts of *Pythium* indicated an effect of treatment one month after fumigation ($P = 0.036$), but not after seven months ($P = 0.313$). Only nonfumigated control treatments had significantly less reduction in *Pythium* counts than those counted from plots treated with any of the five fumigant treatments. However, nonfumigated control plots also experienced a reduction in *Pythium* counts by 60-92% one month after fumigation or by 68-98% seven months after fumigation. No fumigant treatment was significantly different from the conventional application of methyl bromide/chloropicrin. No difference in efficacy was observed between HDPE and VIF of the methyl iodide/chloropicrin treatments.

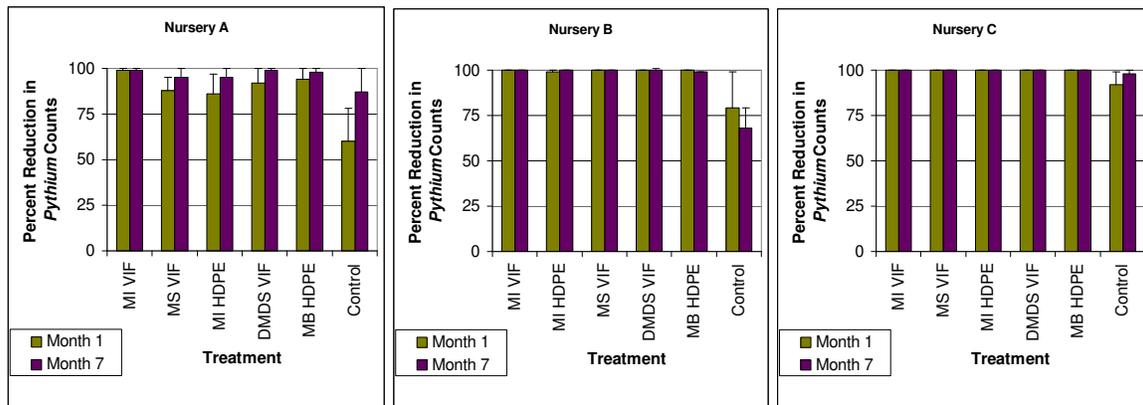


Figure 1. Percent reduction in *Pythium* species counts from dilution plating of fumigant-treated soil at three forest tree nurseries. MI = methyl iodide, MS = metam sodium, DMDS = dimethyl disulfide, MB = methyl bromide, VIF = virtually impermeable film, HDPE = high density polyethylene.

Four hundred fifty isolates of *Pythium* from leaf baits of the three nurseries have been identified on the basis of ITS sequence to date. One hundred eighty nine isolates (42%) were identified as *P. irregulare*, 122 as *P. dissotocum* (27%), 45 as *P. macrosporium* (10%), and

27 as *P. spiculum* (6%). The remaining 15% of the isolates were identified as one of 11 separate species including: *P. cylindrosporum*, *P. folliculosum*, *P. mamillatum*, *P. middletonii*, *P. monospermum*, *P. pachycaule*, *P. rostratfingens*, *P. rostratum*, *P. sylvaticum*, *P. torulosum*, and *P. ultimum*.

DISCUSSION

Sequencing of isolates from soil dilution plating and baiting is in progress. Several species such as *P. irregulare*, *P. macrosporum*, and *P. dissotocum* may more properly be described as species complexes that include several distinct ITS sequences in the nucleotide database at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). These species complexes consist of several species that differ genetically but appear similar morphologically. *Pythium irregulare*, for example, was recently divided into two separate species (*P. irregulare* and *P. cryptoirregulare*) on the basis of AFLP markers and ITS and cox II gene sequences and likely consists of several other species that have yet to be elucidated (Garzon and others 2007). As new information on these species complexes becomes available, further analyses of the isolates from the present study will be conducted.

In May 2009, two-year-old transplants of Douglas-fir were transplanted into each treatment plot. A subset of transplants was assayed for *Pythium* colonization by plating 1-cm-length root pieces on PARP. Most transplants were apparently free of *Pythium* colonization. However, *Pythium* was isolated from transplants from two seedling sources, and these isolates are currently being identified to species. Transplant harvest will occur in October-November 2009, and a final soil and root assay for *Pythium* species will occur at that time.

Other components of this research that are in progress include: 1) comparison of *Pythium* species isolation frequency as a function of isolation method; and 2) pathogenicity tests. Preliminary evidence suggests that recovery frequency of certain *Pythium* species is dependent on the method (dilution plating versus baiting) and on the baits used (rhododendron versus Douglas-fir) used. In addition, greenhouse assays of Douglas-fir germination in soil infested with single spore *Pythium* isolates (from the present study) found that at least 11 species are pathogenic.

This study addresses several key components of *Pythium* damping-off in forest tree nurseries. In addition to evaluating the efficacy of lower doses of alternative fumigants for management of *Pythium*, we are also analyzing *Pythium* species frequency and diversity within three nurseries. Knowledge of *Pythium* species identity is critical for disease management. Some species may not be pathogenic to conifer seedlings, and those that are pathogenic may vary in the amount of damage that they cause. In addition, the relative abundance of each species may play an important role in evaluating economic thresholds. Results from this study are expected to detail the efficacy of lower doses of alternative fumigants for management of *Pythium* species and to elucidate the etiology of *Pythium* species associated with damping-off in forest tree nurseries.

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THE OCCURRENCE OF PEST AND DISEASE OF *GMELINA ARBOREA* FROM CLONAL AND SEED MATERIAL IN THE NURSERIES: A CASE STUDY IN EAST KALIMANTAN, INDONESIA

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ABSTRACT

Gmelina arborea Roxb. (Family Verbenaceae) is a fast growing tree frequently planted in plantations to produce wood for light construction, craft, decorative veneers, pulp, fuel and charcoal. Tree plantations using clonal material have many advantages, particularly on maintaining consistent production of the same genotypes over time. Due to genetic uniformity and narrow genetic diversity, clonal plantations are presumed to be more vulnerable to pests. The purpose of this study was to compare the occurrence of potential pests on *G. arborea* produced from clonal material and seed in the nurseries. The following parameters were evaluated: the incidence (I) and severity (S) of pest species. Results of the study indicated that there were three pathogens potentially causing disease in the nursery: *Pythium* sp., *Colletotrichum* sp. and *Sclerotium* sp. *Pythium* sp. and *Colletotrichum* sp. caused leaf blight and leaf spot disease only on seedlings (S=40%, I=80% and S=57%, I=44.5%, respectively). *Sclerotium* sp. was associated with stem base rot and occurred on the clonal material only (S=60%, I=30%). Beehole borer, identified as *Xyleutes ceramica*, attacked the stem of three month seedlings and clonal material (I= 47.04 % and 44.40%, respectively). *Alcides gmelinae* attacked only seedlings from clonal material (I=2%). Clonal stock material are not always more prone to disease and insect attack than seedlings. The incidence of pests was more influenced by nursery conditions and behavior of the pathogen or insect than the type of *G. arborea* stock.

Key words: pest, clonal, seedling, *Xyleutes ceramica*, *Gmelina arborea*, *Alcides gmelinae*

INTRODUCTION

Gmelina arborea Roxb. (Family Verbenaceae) is a fast growing tree frequently planted in plantations to produce wood for light construction, craft, decorative veneers, pulp, fuel and charcoal. This species originated in an area of South and Southeast Asia from Pakistan and Sri Lanka to Myanmar. It has been widely planted in Southeast Asia Countries including Bangladesh, Myanmar, Thailand, Southern China, Vietnam, Indonesia and Philippines (Jensen 1995). In order to get the potential productivity and the uniformity of product, in 1995 some forest plantation in East Kalimantan, Indonesia started to develop clonal forestry practices, particularly using shoot cuttings of *G. arborea*. According to Zobel (1992) clonal plantation are at high risk to serious damage by pests, especially when only a single clone is planted. Park (2002) also notes one major concern about deploying clones in plantations is that a narrow genetic base may make clone plantations more vulnerable to disease and insects

than trees in a natural forest, thus leading to plantation failure. However, careful management of clone numbers and the way they are interplanted can minimize pest problems (Evans 2001). Roberds and Bishir (1997) suggest that using 30-40 unrelated clones will generally provide security against catastrophic failure. In order to understand the potential pests that may occur, on trees from clonal and seed sources, earlier evaluation at the nursery is required.

METHODS

Research was conducted in the Sebulu plantation forest, which is located in Samarinda, East Kalimantan, Indonesia. Study plots of 10 x 10 m were established in clonal and seedling stock in the nursery. 10 sub-sample plots were arbitrarily set up in each plot. Inventory and evaluation of insects and diseases were made on the clones and seedlings in the nursery according to the following criteria:

1. Severity, which based on the seriousness of symptom (Severe, Medium, and Low) and the average severity of the diseases was modified by Sharma and others (1985) formula as followed. 0% = nil, up to 25% affected = low, > 25% - 50% affected = medium, > 50%-100% affected = severe.
2. Incidence, which based on percentage of trees affected. The incidence of the pest and or disease in a plot was rated as follows: widespread = more than 75%, very common = 50 < 75%, common = 25-<50%, occasional = 10<25%, rare = less than 10%.

Disease occurrence was evaluated using severity and incidence criteria, while insect pests were evaluated using incidence criteria only.

RESULTS AND DISCUSSION

The occurrence of insects and diseases on seedlings from clonal material and seedlings of *G. arborea* are listed in Table 1. Leaf blight and leaf spot disease of *G. arborea* were common in the nursery and caused death of seedling. *G. arborea* from shoot cutting material was never affected by leaf disease, which may be due to maturity of their leaves when transplanted to the nursery. Clonal material may be able to escape the succulent phase that is generally more susceptible to leaf disease (Rahayu and others 1999).

Table 1. The occurrence of insects and disease on *Gmelina arborea* nursery stock from shoot cuttings and seed.

Pest	Causal agent	Clonal stock ³		Seedling stock ³	
		S (%)	I (%)	S (%)	I (%)
Leaf blight ¹	<i>Pythium</i> Sp.	0	0	40	80
Leaf spot ²	<i>Colletotrichum</i> Sp.	0	0	57	44.5
Stem base rot ²	<i>Sclerotium</i> Sp.	60	30	0	0
Bee hole borer ²	<i>Xyleutes ceramicus</i>	0	44	0	47
Shoot cutting ²	<i>Alcides gmelinae</i>	0	2	0	0

¹ Stock under 3 weeks

² Stock between 3 to 9 weeks

³S = Severity and I = Incidence of pests

Stem base rot was never seen in the seedlings. According to Mordue (1974), *Sclerotium* sp. on *G. arborea* is commonly present on vegetative than generative propagation material. This may be related to the characteristics of *Sclerotium* sp. as a facultative parasite able to develop sclerotia that can survive for long time in the soil. In addition, conditions that surround nurseries may be more suitable for developing stem base rot from *Sclerotium* sp. due to humidity in relation to the more rapid growth of clonal material.

Alcides gmelinae (Fig. 1) generally attack young trees; however, this insect may have moved to the nurseries from plantations located close by. Currently, physical control by picking the insect up manually is still possible due to the low incidence of this pest (2%).

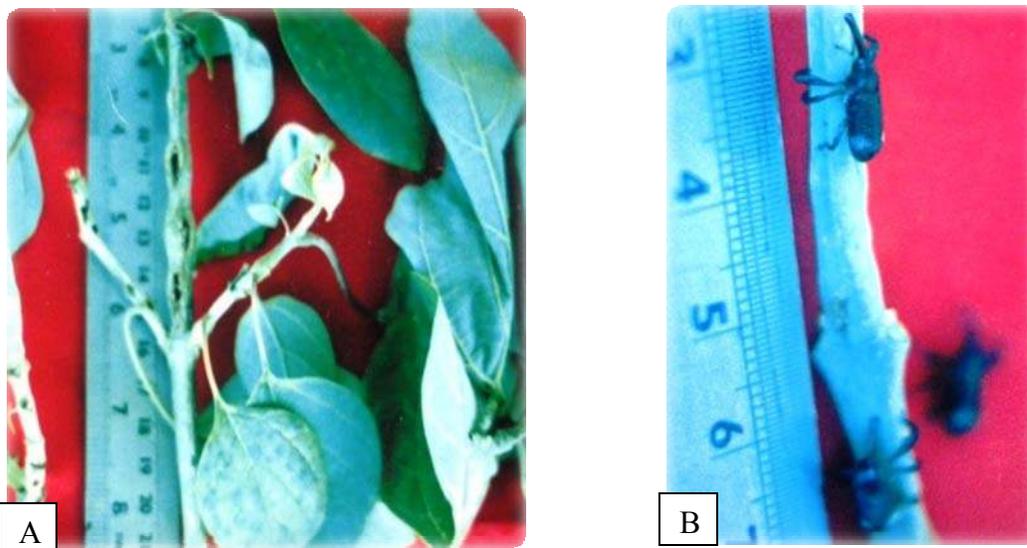


Figure 1. A) Swollen and intensive hole on the shoot, caused by B) *Alcides gmelinae* on *Gmelina arborea* seedling.

Beehole borer, *Xyleutes ceramicus* (Fig. 2) is a rather new insect on *G. arborea*. Previously the larvae stage of this insect was known to attack young teak plantations (Intari, 1975); preferably clonal teak plantation (Rahayu 2001). The incidence of beehole borer on both clonal cuttings and seedlings are not significant. In this case, the genotype of clone material and seedling family is the same, which may explain the lack of a significant difference.

The results of this study indicate that nursery stock from vegetative material, specifically from clonal material, are not always more prone than seedlings to disease and insect attack. The incidents of pests on *G. arborea* appeared to be dependent on the nursery condition, stage and age of nursery stock, and behavior of the insect or pathogen in association with the species.



Figure 2. Larvae of Bee hole borer, *Xyleutes ceramicus* inside the stem of a *Gmelina arborea* seedling.

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DISTRIBUTION AND GENETIC DIVERSITY OF ROOT-ROT PATHOGEN *NEONECTRIA MACRODIDYMA* IN FOREST NURSERY

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ABSTRACT

The aim of this study was to assess fungal communities in fine living roots of *Pinus sylvestris* seedlings in a forest nursery and to determine genetic diversity and spatial distribution of common fungal symbiont. Root systems of 100 seedlings were collected using 1.5m x 1.5m grid design in the area of 225 m². From each root system, 20 individual fine roots were sampled randomly, surface sterilized and plated on MMN media for fungal isolation. Isolation yielded 606 pure cultures, which using morphological and molecular methods, were identified as 71 distinct taxa. Root-rot pathogen *Neonectria macrodidyma* was the most common isolate and comprised 20.3% of the total fungal community. As ITS rDNA sequences were 100% identical for all strains, polymorphism of intergenic spacer (IGS) of rDNA was studied by means of double restriction digestion of 3.8 kbp-long PCR amplicons to determine genetic diversity of *N. macrodidyma* isolates. Restriction digestion showed that among 123 strains of *N. macrodidyma* only two distinct IGS types were present at the frequency 40:83. Mapping data and estimates on spatial distribution using nearest neighbor method revealed overlapping occurrence and even distribution of both IGS types in the study area. Results of this study indicate that *N. macrodidyma* is commonly associated with fine living roots of pine seedlings, is largely disseminated by vegetative means of local genotypes and has even distribution in forest nursery soils. Furthermore, in living roots *N. macrodidyma* is likely present as dormant propagules but under favorable conditions it may develop rapidly and have a significant negative effect on plant health and productivity.

INTRODUCTION

In forest nurseries, seedling production may often be limited by root diseases caused by fungal pathogens. Typical symptoms of root infections in conifer seedlings are stunted growth, discoloration of needles and partial or complete death of the root systems (Lilja and others 1992), which essentially may lead to a significant decrease in plant quality. In some cases, due to the intensive management practices, aboveground disease symptoms in the nursery may be absent but seedlings, infected with root pathogens, may exhibit reduced survival rates following their outplanting in the field.

Fungi from the genera *Fusarium*, *Neonectria*, *Rhizoctonia*, and *Pythium* have been reported as the main causal agents of the root dieback in forest nurseries (Galaaen and Venn 1979, Lilja and others 1992, Kope and others 1996, Lilja and Rikala 2000, Menkis and others 2006). A majority of these fungi are considered to be opportunistic, necrotic pathogens that produce toxins to invade and kill the plant tissues (Unestam and others 1989, Beyer-Ericson

and others 1991). They may often act as saprotrophs while being attached to the surface of living roots, but stress and reduction in seedling vitality may induce a rapid pathogenic response by these fungi (Unestam and others 1989). Although there is a substantial amount of information on incidence of root pathogens in bare-root forest nurseries, information on genetic diversity, distribution on the local scale and potential importance of those fungi on asymptomatic seedling roots is largely unavailable. Such information is also of practical importance allowing further optimization of management practices in forest nurseries.

In this study, fungi associated with seedling roots were assessed in a confined nursery plot using systematically sampled seedlings of *Pinus sylvestris*. Combining fungal isolation into pure culture and molecular fingerprinting, we determined both communities of root inhabiting fungi and genetic diversity and spatial distribution of common fungal symbiont.

METHODS

The study site was located in a forest nursery situated in the vicinity of the Baltic Sea coast in western Lithuania. This nursery produces seedlings using standardized bare-root cultivation in beds. Two-year old seedlings of *Pinus sylvestris* were sampled after the growing season in October 2007. The sampling area was 225 m² in size and included four adjacent beds each 1.5 m width and 37.5 m long. In total, 100 seedlings were sampled using a systematic grid design at a spacing of 1.5 m x 1.5 m. Seedlings were gently excavated to preserve fine roots, individually labeled, packed into plastic bags, transported to the laboratory and kept at 4°C until analysis.

The isolation of fungi into pure culture was attempted from 2000 individual root tips, which were obtained by randomly sampling 20 individual root tips from each root system of 100 plants. Before isolation, root tips were placed in 10 × 20 mm net bags (mesh size 0.2×0.2 mm), sterilized in 33% hydrogen peroxide for 30 s, and then rinsed three times in sterile deionised water. About ten tips per each Petri dish were plated onto modified Melin Norkrans medium (Marx 1969) and incubated at room temperature in the dark. Dishes were checked daily and any newly growing mycelia was immediately subcultures onto fresh agar media. Isolated cultures were examined under a microscope (Carl Zeiss Axioplan, Oberkochen, Germany) equipped with 10× ocular and 25× long distance objective magnification, and grouped into mycelial morphotypes.

For identification, the internal transcribed spacer of the fungal ribosomal DNA (ITS rDNA) was sequenced for representatives of each mycelial morphotype using primers ITS1F and ITS4 (White and others 1990). Extraction of DNA, amplification and sequencing followed established methods described by Rosling and others (2003). Raw sequence data were analyzed using the SeqMan version 5.01 software from DNASTAR package (DNASTAR, Madison, WI, USA) and BioEdit version 7.0.5.2 (Hall 1999). Databases at GenBank (Altschul and others 1997), UNITE (Koljalg and others 2005) and at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala were used to determine the identity of sequences.

Genetic diversity of fungal isolates was studied by using PCR based restriction fragment length polymorphism (PCR-RFLP) of intergenic spacer (IGS) of the nuclear ribosomal DNA. Amplification of the entire IGS region, situated in between 26S and 18S genes of rDNA, was done by primer pair LR20R and SR7. Description of those primers is available at <http://www.lutzonilab.net/primers/index.shtml>. Double digestion of amplified PCR products was done by using restriction enzymes HinfI and HhaI (Fermentas Life Sciences, Germany) according to the manufacturer's recommendations. Restriction fragments were separated by electrophoresis on 1% agarose gels (Agarose D1, Conda, Spain) in 1x SB buffer (Brody and Kern 2004) for 3h at 150V. The gels were stained with ethidium bromide and obtained images were analyzed by Quantity One version 4.6.3 (Bio-Rad laboratories, CA, USA) software. Clark – Evans nearest neighbor statistics (Clark and Evans 1954) were used to estimate whether distribution of isolated fungi in nursery plot was random, even or clustered.

RESULTS AND DISCUSSION

Of 2000 fine roots used for isolation of fungi into pure culture, 606 (30.3%) resulted in fungal growth, and the remaining 1394 (69.7%) either remained sterile following surface sterilization or were colonized by bacteria and/or rapidly growing fungi from neighboring roots plated in the same dishes. Therefore, isolation in this study yielded 606 pure cultures, which following morphological and molecular identification, were recognized as 71 distinct taxa. Of those, 50 (70.4%) were identified at least to genus level. For unidentified taxa, only 4 (5.6%) could be matched to ITS rDNA sequences available in the databases and 17 (23.9%) showed unique sequences. Of the total isolated fungal community, 89.3% were ascomycetes and deuteromycetes and 10.7% were basidiomycetes. Frequently isolated fungi were ascomycetes and deuteromycetes *Neonectria macrodidyma* (20.3%), *Phialocephala fortinii* (13.5%), Unidentified sp. PM29C (4.8%) and basidiomycete *Hebeloma cavipes* (4.6%).

Neonectria macrodidyma appeared to be the most commonly isolated taxon from the healthy-looking root tips of bareroot nursery cultivated pine seedlings. This indicated that *N. macrodidyma* play an important role in determining plant health and productivity and may be of great economic importance. To gain more specific information on this species, both genetic diversity of the isolates and their spatial distribution in the confined nursery plot was studied. Firstly, we identified sequence variation within ITS rDNA for 123 isolates of *N. macrodidyma*. As ITS rDNA sequences were 100% identical for all strains, polymorphism of IGS rDNA was studied by means of double restriction digestion of PCR amplicons. In *N. macrodidyma* a total size of amplified IGS rDNA region was ca. 3.8kb in size. Restriction digestion of amplified products showed that among all strains of *N. macrodidyma* isolated in this study, only two distinct IGS types were present at the frequency 40:83. Mapping data for isolates of each IGS type and estimates on their spatial distribution revealed that both IGS types were intermingled and evenly distributed in the nursery plot.

Neonectria macrodidyma was recently described as a new species (Halleen and others 2004), commonly associated with black foot disease of wine grapes in a wide geographic area (Alaniz and others 2007, Auger and others 2007). Disease symptoms included drying and

dying shoots, abnormal development and necrosis of roots, black discoloration of the wood and overall reduction in root biomass. In our previous studies, this species was also commonly isolated from healthy-looking and diseased roots of nursery grown conifer seedlings (Menkis and others 2005, Menkis and others 2006). This suggests that *N. macrodidyma*, similar to many other *Neonectria* spp., is a plant pathogen well adapted to a wide range of hosts and habitats.

The results of the present study confirmed an earlier observation on intimate association of *N. macrodidyma* with living tree roots. More importantly, information on both limited genetic diversity of isolates and their even distribution in the nursery plot was acquired. The latter results suggests that *N. macrodidyma* is largely disseminated by vegetative means of local genotypes and that soil cultivation practices is likely contribute to dissemination of this species in the forest nursery soils. Often isolation of this species from the asymptotic fine roots may further suggest that in living roots *N. macrodidyma* is present as dormant propagules, but under favorable conditions it may develop rapidly and have a significant negative effect on plant health and productivity.

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PATHOGENICITY AND MANAGEMENT OF *TYLENCHORHYNCHUS CLAYTONI* IN SOUTHERN USA FOREST NURSERIES

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ABSTRACT

The stunt nematode, *Tylenchorhynchus claytoni*, has been a recognized problem in pine nurseries in the southern USA since the 1950's. Pathogenicity tests for *T. claytoni* on loblolly (*Pinus taeda*) and slash pine (*P. elliottii*) seedlings found that populations of 125 nematodes or greater per 100 cc soil caused a reductions in root volume. Host range tests of common cover crops found that sorghum-sudan grass (*Sorghum bicolor* 'SG Ultra'), rye (*Secale cereale* 'Elbon'), corn (*Zea mays* 'Roundup Ready'), ryegrass (*Lolium multiflorum* 'TAM 90'), oats (*Avena sativa* 'Mora'), buckwheat (*Fagopyrum esculentum* 'Mancan'), velvetbean (*Mucuna pruriens*), Kobe lespedeza (*Lespedeza striata* 'Kobe'), bicolor lespedeza (*Lespedeza bicolor*), and purple nutsedge (*Cyperus rotundus*) are suitable hosts for the stunt nematode. Pearl millet (*Pennisetum americanum* 'ET-300') and brown top millet (*Panicum ramosum* 'DW-01') were found to be poor hosts and had the lowest population densities of the stunt nematode among the cover crops. Only the fallow treatment performed better. A subsequent two year field test of sorghum-sudangrass hybrid 'Sugar Graze', pearl millet hybrid 'Tifleaf 3', and fallow found that the use of pearl millet as a cover crop greatly reduces the population densities of the stunt nematode in infested fields, and did not differ statistically from the fallow treatment after the first year.

INTRODUCTION

Loblolly (*Pinus taeda*) and slash pine (*P. elliottii*) are known hosts for the stunt nematode *Tylenchorhynchus claytoni* (Ruehle 1966). Although stunting of loblolly pine seedlings has been associated with high population densities of *T. claytoni* (Hopper 1958, Ruehle 1969), pathogenicity testing has only been performed on longleaf pine (*P. palustris*) (Ruehle 1973). The population densities at which *T. claytoni* can damage loblolly and slash pine seedlings remains a basic gap in our understanding of the impact of this nematode.

In southern forest tree nurseries, cover crops are alternated with tree seedlings for maintaining organic matter and soil stabilization as well as a reduction of pests (Boyer and South 1984). The use of non-host and poor-host cover crops, as well as fallow, has shown promise for controlling a needle nematode (*Longidorus americanus*) and a stunt nematode (*Tylenchorhynchus ewingi*) in pine nurseries (Fraedrich and others 2003, Cram and Fraedrich 2005, Fraedrich and others 2005). Host-range testing may help forest nursery managers identify non- or poor-host cover crops to be used in a pest management program for *Tylenchorhynchus claytoni*. Growth chamber tests provide a practical and efficient means of

screening a wide range of species for host status, but field testing is necessary to determine crop performance and nematode population changes over time under operational conditions.

A southern forest nursery historically had problems with smaller loblolly pine seedlings in some fields during the second year of production following fumigation. A 2005 survey of a loblolly pine field in the first pine production following fumigation revealed *T. claytoni* increased from a field average of 0.17 (April) to 126 (December) per 100 cc soil. By April 2006 the population density of *T. claytoni* averaged 402 nematodes / 100 cc soil and ranged as high as 788 nematodes / 100 cc soil (Fig. 1). A cover crop field test was initiated in this field using pearl millet, a cover crop determined to be a very poor to non-host for *T. claytoni*.

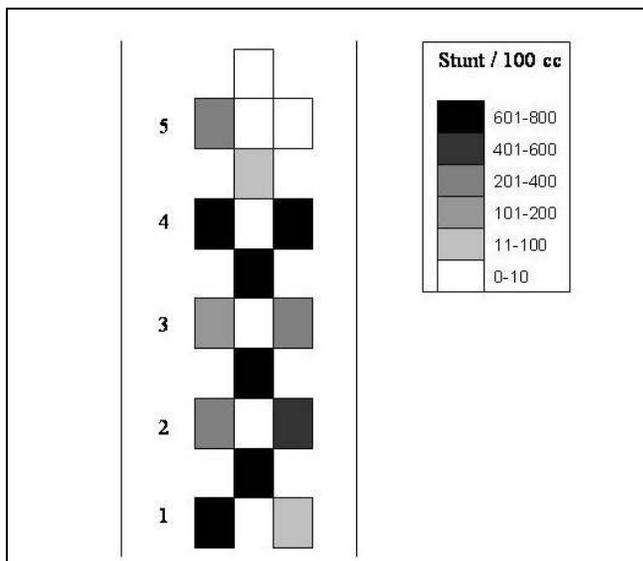


Figure 1. Field diagram of stunt nematode populations by plot in April 2006.

METHODS

Pathogenicity Test

The effect of *T. claytoni* population density on loblolly and slash pine seedlings was evaluated in a growth chamber experiment. Containers were filled with approximately 400 cc of a loamy sand soil that was microwaved in 2000 g batches for eight minutes. Containers were planted with five germinating loblolly pine seeds. Nematodes were reared on roots of loblolly pine seedlings and subsequently extracted with Baermann funnels (Shurtleff and Averre 2000). Nematodes were added to containers at rates of 0, 500, 1000, 2000 and 4000 individuals/container, and there were four replications for each nematode dose. Containers were placed in growth chambers at 25°C with a 14 hour photoperiod and watered every 1 to 3 days, as needed. After 10 weeks, plants were removed from the containers and placed in tap water for 15-30 minutes to remove soil and nematodes from plant roots. These nematodes were washed back into the soil sample using a 325 mesh screen, and soil samples were mixed thoroughly. Roots were placed in plastic bags and stored at 6°C. Root volume (cm³) was calculated by WinRHIZO Version 2003b scanning system. Nematodes were extracted from

100 cc of soil using the centrifugal-flotation method (Shurtleff and Averre 2000). The relationship between the initial *T. claytoni* dose and root volumes was determined by regression analysis using a nonlinear, negative exponential model. The analysis was conducted using the regression analysis package of SigmaPlot (Version 8.0). The criteria for fit of the model were based on the mean square error (MSE), r-square values, and the significance of the overall regression.

Host Range Tests

Cover crops, typically used in the southern USA forest nurseries, were tested for their suitability as hosts for *T. claytoni*. The first host test included sorghum-sudan (*Sorghum bicolor* 'SG Ultra'), rye (*Secale cereale* 'Elbon'), corn (*Zea mays* 'Roundup Ready'), ryegrass (*Lolium multiflorum* 'TAM 90'), oats (*Avena sativa* 'Mora'), pearl millet (*Pennisetum americanum* 'ET-300'), and brown top millet (*Panicum ramosum* 'DW-01'). The second host test included buckwheat (*Fagopyrum esculentum* 'Mancan'), velvetbean (*Mucuna pruriens*), Kobe lespedeza (*Lespedeza striata* 'Kobe'), and bicolor lespedeza (*Lespedeza bicolor*), as well as purple nutsedge (*Cyperus rotundus*); a common southern weed. Loblolly pine and bare fallow treatments were also included as controls in each test.

In each host test, soil with a loamy sand texture was microwaved for 8 minutes in 2000 g batches, and containers were filled with 1600 cc of soil. There were four replications (containers) for each treatment and 5 plants were established in each container (except the fallow containers). Stunt nematodes were extracted from stock cultures using a Baermann funnel method (Shurtleff and Averre 2000). In host test 1 and each treatment container was infested with 1,000 nematodes. In host test 2 containers were infested with 500 nematodes. Containers were placed in a growth chamber at 25°C with a 15 hr photoperiod. Stunt nematode population densities were determined after 12 weeks in both tests. Nematodes closely associated with roots were extracted by placing roots in approximately 1 liter of water for 15 minutes, and then extracting nematodes on a 325 mesh screen. These nematodes were then placed in the soil which was mixed well before removing 100 cc of soil for determination of nematode population densities. Nematodes were extracted from soil samples using the centrifugal flotation method (Jenkins 1964). Roots were dried for 48 hours at 80°C and dry weights subsequently determined.

The final population densities of nematodes were compared among treatments in each host range test using an ANOVA and Tukey's HSD test. Data were transformed with the $\log_{10}(x+1)$ transformations prior to analysis, but only nontransformed values are presented in tables.

Field Cover Crop Trial

The soil type for the field trial was a sandy loam soil in the Wagram Sand soil series. The field had been fumigated in the fall of 2004 with methyl bromide (67%) and chloropicrin (33%) at a rate of 350lb/ac (392.9 kg/ha), and then sown with loblolly pine seeds in 2005. In the spring of 2006, a test was established with sorghum-sudangrass hybrid 'Sugar Graze', pearl millet hybrid 'Tifleaf 3', and fallow treatments. The 'Tifleaf 3' cultivar was selected because this cultivar was readily available to the nursery, and the nursery had just begun using this cultivar operationally.

Each treatment had five replications and the study was established as a randomized complete block design. The checkerboard pattern of plots was created by dividing the field into three 3 m widths by ten 15.24 m lengths and leaving sections between the treatment plots as fallow buffers (Fig. 1). Sorghum-sudangrass and pearl millet were sown on April 25, 2006 and again on May 4, 2007. The sowing rate was 33.6 kg/ha for sorghum-sudangrass and 16.8 kg/ha for pearl millet. The study area was watered with approximately 2.5 cm of water per week for 12 weeks. One application of granular ammonium nitrate at a rate of 57.16 kg/ha of N was applied after 6 weeks. Fomesafen sodium (Reflex®) and lactofen (Cobra®) were each applied at 2.3 l/ha in fallow areas at sowing. Glyphosate (Gly 4plus) was added as a 5% solution as needed during the growing season.

Soil samples were obtained in April (prior to sowing), May, June, September, and November of each year. The soil was systematically sampled from the center of each treatment plot and consisted of 6 corings taken to a 15 cm depth. Composite soil samples were mixed and nematodes were extracted from a 100 cc sub-sample using the centrifugal-flotation method (Shurtleff and Averre 2000). The percent organic matter for soil samples collected in April 2007 and 2008 was determined using the Dumas combustion elemental analysis at the University of Georgia's Soil Biology Laboratory in the Institute of Ecology.

Nematode population densities were compared among cover crop treatments by an ANOVA using the PROC GLM procedure of SAS (the SAS System for Windows), and mean separation was performed by Tukey's HSD test. Block 5 was removed from the analysis due to low initial nematode population. Data were transformed with the square root ($x + \frac{1}{2}$) transformations prior to analysis, but only nontransformed values are presented in graphs.

RESULTS

Loblolly and slash pine root volume decreased with respect to the initial populations of the stunt nematode *T. claytoni* (Figure 2). The relative fit of the negative exponential model, based on the R^2 values and MSE's, was slightly better for loblolly pine (MSE=0.0027; $R^2=0.92$) than slash pine (MSE=0.0073; $R^2=0.82$). Initial population densities as low as 500 nematodes per 400 cc soil (125/100 cc) greatly reduced the root volume of both pine species. The level of damage was similar for all doses of the stunt nematode.

Host Range Tests

An evaluation of common cover crops as hosts for the stunt nematode found that pearl millet was the poorest host, followed by Brown Top Millet (Table 1). All other crops and purple nutsedge were hosts for the stunt nematode *T. claytoni* (Tables 1 and 2). The fallow treatment had the lowest number of stunt nematodes in both tests.

Field Cover Crop Trial

The stunt nematode *T. claytoni* was the predominant nematode species isolated from treatment plots in the field study. Some plots also had stubby-root nematodes, *Paratrichodorus minor*, and predacious nematodes (*Mylonchulus* sp., *Mononchus* sp.). One other plant parasitic nematode, *Paratrichodorus porosus*, was found during the second year

of the field test in the sorghum-sudangrass treatments only. Population densities of *P. porosus* were usually less than 50 nematodes per 100 cc soil.

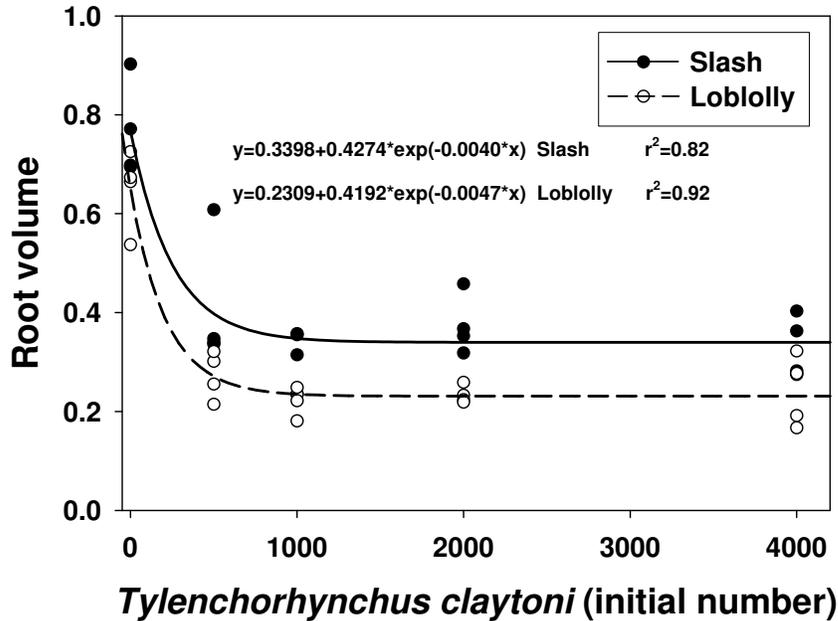


Figure 2. Relationship between initial population of stunt nematode (*T. claytoni*) and root volume (cm³) of seedlings after 10 weeks.

Table 1. Population densities of stunt nematodes in containers with various cover crops 12 weeks after infestation with 1000 stunt nematodes/container (1600 cc).

Plant species	Stunt nematodes per 100 cc soil ⁺	
Rye grain ('Elbon')	7393	a
Loblolly Pine	6753	a
Corn ('Roundup ready')	3545	ab
Sorghum sudan ('Ultra')	1905	bc
Oats ('Mora')	1478	c
Rye grass ('TAM-90')	949	c
Brown Top Millet ('DW01')	319	d
Pearl Millet ('ET-300')	148	d
Fallow	35	e

⁺Means followed by the same letter do not differ significantly (alpha=0.05) according to Tukey's HSD test. Logarithmic transformation of nematode counts performed before analysis. Data analyzed as a randomized complete block design

Table 2. Population densities of stunt nematodes in containers with various crop and weed species 12 weeks after infestation with 500 stunt nematodes/container (1600 cc soil).

Plant species	Stunt nematodes per 100 cc soil ⁺	Stunt nematodes per container ⁺
Buckwheat ('Mancan')	798 a	12760 a
Velvetbean	615 a	9840 a
Loblolly Pine	318 a	5080 a
Kobe Lespedeza	243 a	3880 a
Bicolor Lespedeza	159 a	2540 a
Purple nutsedge	135 a	2160 a
Fallow	5 b	80 b

⁺Means followed by the same letter do not differ significantly (alpha=0.05) according to Tukey's HSD test. Logarithmic transformation of nematode counts performed before analysis.

Over a two year period the average population densities of the stunt nematode decreased significantly in the fallow and pearl millet cover crop treatments (Fig. 3). The population of stunt nematodes in the fallow treatment fell below 100 individuals/100 cc soil by the end of the first year. The number of stunt nematodes within the pearl millet treatment did not fall below 100 individuals/100 cc soil until April of the second year. An examination of the average population densities of the stunt nematode in the sorghum-sudangrass over the two year study indicated nematode densities decreased during August and September and increased greatly in the winter and spring.

The population densities of the stubby-root nematode *P. minor* were greater in the sorghum-sudangrass and pearl millet plots than in the fallow, although the densities remained under 100 nematodes/100 cc soil during the two years (Fig. 4). Population densities of predacious nematodes remained very low (0.5 - 17.5 predators/100 cc) throughout the two year study and did not appear to be affected by season.

Soil organic matter was similar for the sorghum-sudangrass and the pearl millet treatments by April of both years (Table 3). Pearl millet significantly improved the percent organic matter in the soil as compared to the fallow treatment.

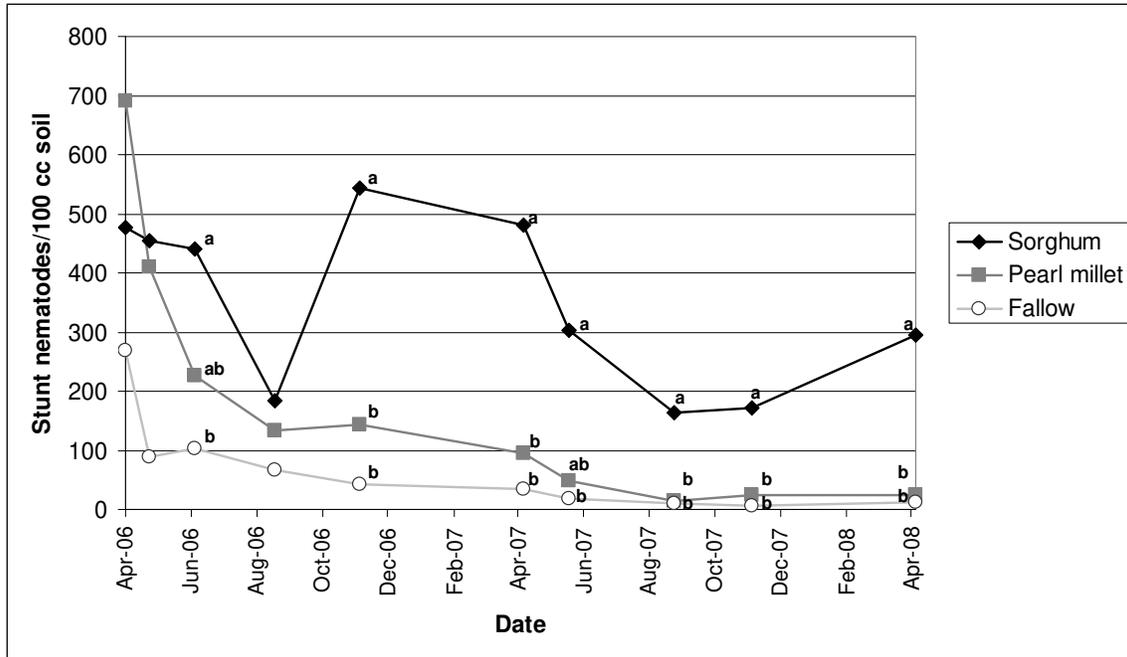


Figure 3. Relationship between stunt nematode (*T. claytoni*) population densities and cover crop treatment over 2 years. Means were transformed by square root of $(x + \frac{1}{2})$; treatments in block 5 were removed from analysis due to low initial nematode pop. Data points followed by a different letters by date are significantly different using Tukey's HSD test (Alpha 0.05).

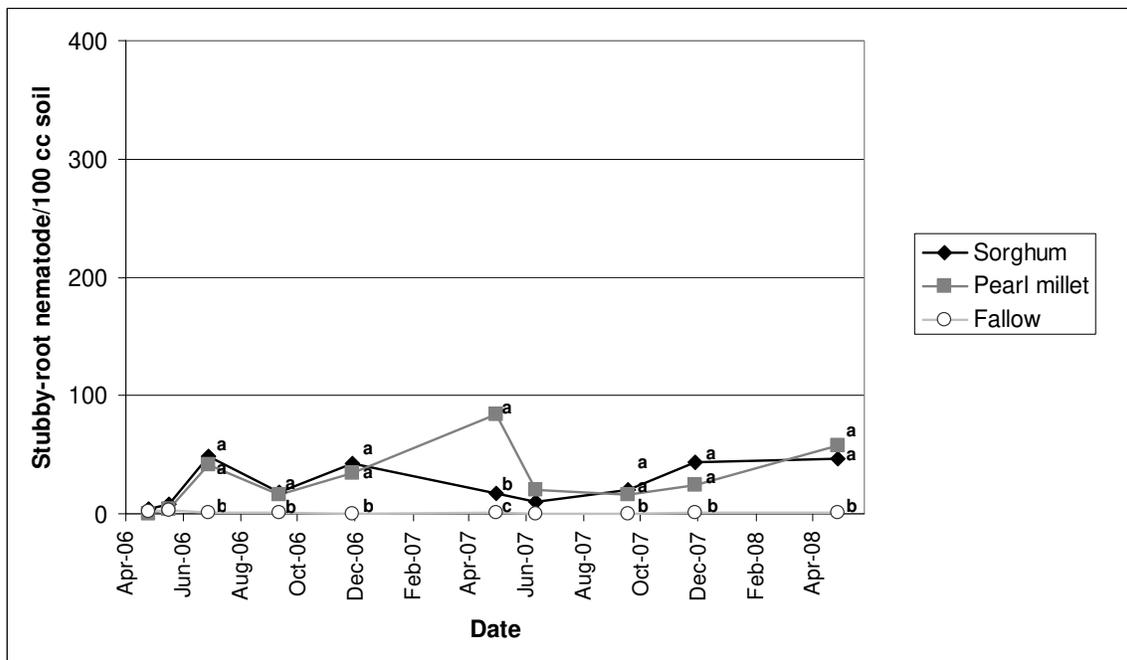


Figure 4. Relationship between stubby-root nematode (*M. minor*) population densities and covercrop treatment over 2 years. Data transformed by square root of $(x + \frac{1}{2})$; treatments in block 5 were removed from analysis due to low initial nematode pop. Data points followed by a different letters by date are significantly different using Tukey's HSD test (Alpha 0.05).

Table 3. Percent carbon as a measure of organic matter in soil by cover crop after the first and second year of treatment.

Cover crops	April 2007		April 2008	
	Percent carbon ⁺		Percent carbon ⁺	
Sorghum-sudangrass	0.79	ab	0.89	a
Pearl millet ('Tifleaf 3')	0.89	a	0.86	a
Fallow	0.57	b	0.51	b

⁺Means within columns followed by the same letter do not differ significantly ($\alpha=0.05$) according to Tukey's HSD test.

DISCUSSION

The results of the dosage response trials indicate that the stunt nematode, *T. claytoni*, can directly cause stunting of loblolly and slash pine root systems when the nematode is present during seed germination and early growth of young seedlings. The impact of the stunt nematode on loblolly and slash pine seedlings during the first 10 weeks in this study is similar to what occurs on these pine species in nursery beds (Hopper 1958, Ruehle 1973). The results of our pathogenicity test suggest that the high densities of the stunt nematodes present in the field study on April 25, 2006 would probably have led to areas of seedling damage and losses had the nursery produced pine seedlings in the field as they would have under their typical crop rotation.

The nursery typically alternated sorghum and grain rye with pine crops, all of which are good hosts for *T. claytoni* according to our results. Continuously growing crops that are hosts for a nematode can lead to damaging populations (Dropkin 1989, Cram and others 2003). The use of fallow or alternating hosts with non-hosts can help managers control plant-parasitic nematodes (Vargas-Ayala and Rodriguez-Kabana 2001, Fraedrich and others 2005). The host range test results for the population densities of *T. claytoni* with various cover crops were similar to the host range tests for another stunt nematode, *T. ewingi* (Cram and others 2003).

The velvetbean species tested, *M. pruriens*, has shown some promise as a less favored host than sorghum-sudangrass for the stunt nematode in Florida (Crow and others 2001). Extracts from velvetbean stems and roots have also been shown to have nematicidal effects on a root-knot nematode when tested under laboratory conditions (Zasada and others 2006). Our results indicate that this species of velvetbean is a good host for the stunt nematode *T. claytoni*; however, we did not test the potential toxic effects of this species on nematode populations after plant parts are incorporated into the soil. More research on velvetbean is warranted before it is entirely ruled out as a control option for nursery fields infested with the stunt nematode.

The field test of the hybrid pearl millet cultivar 'Tifleaf 3' showed that this cultivar is not a host for the stunt nematode *T. claytoni* and appears to be a good alternative to fallow to decrease nematode populations in fields. Other pearl millet cultivars have also been found to be resistant to a variety of plant-parasitic nematodes, including *P. minor*, *Meloidogyne* spp.,

Belonolaimus longicaudatus, and *Pratylenchus brachyurus* (Timper and others 2002, Timper and Hanna 2005).

The low levels of stubby-root nematodes in the sorghum-sudangrass field plots could be the result of many factors including less favorable environmental conditions and competition by stunt nematodes. The densities of stubby-root nematodes in the field may have been too low to expect substantial damage on loblolly or slash pine seedlings (Ruehle 1969); additional work is needed regarding the affect of this plant-parasitic nematode on pines.

The predacious nematodes (*Mylonchulus* sp., *Mononchus* sp.) appeared to have no impact on the stunt nematode population as their densities did not change over time. The low population of predators and lack of effect on other nematodes has been noted in other studies that have monitored these nematodes (MacGuidwin and Layne 1995, Farris and others 1996). Predacious nematodes are only one component of the organisms that control plant parasitic nematodes, and it is possible that they are not efficient control agents or even predators of the stunt nematode.

The threshold population density of stunt nematodes that young loblolly and slash pine seedlings can tolerate without stunting remains unknown, but seedling size can be significantly reduced at 125 stunt nematodes/100 cc as indicated by the pathogenicity test. Although population levels of nematodes are lowered significantly by pearl millet in one year, it may take two years to get population densities sufficiently low that they will not damage pine seedlings. Nurseries that use a 2:1 rotation of seedling production to cover crops may be better off using fallow (or a combination of organic matter treatments and fallow). Unfortunately stunt nematode populations in the fallow treatments were not reduced to zero in all plots over the 2 years of this study. Populations of *T. claytoni* can explode quickly on hosts because of its relatively short lifecycle (31 to 38 day) (Wang 1971) and production of 1-15 eggs per female (Krusberg 1959). Even with the use of soil fumigation to control nematodes (Johnson and Feldmesser 1987, Dropkin 1989), the population densities can rebound and damage the second production crop (McKenry and Thomason 1976, Sipes and Schmitt 1998, Fraedrich and Dwinell 2005). Fields that have stunt nematodes may not be able to have successive pine crops without the use of a fumigant before each crop. Other options depend on a nurseries land base and access to organic amendments. Managers may consider alternative cropping strategies, such as a 1:1 rotation of pine with fallow (including organic amendments if needed).

Crop rotations with hardwoods, pine and cover crops may also be possible. Associations between the stunt nematode and stunting of hardwood tree seedlings have not been well documented (Ruehle 1968). The only known nonhost hardwood species identified to date is sweetgum (*Liquidambar styraciflua*), while yellow-poplar is a very poor host (Ruehle 1971). Hardwoods tolerant to stunt nematodes could be used in a rotation with a pine crop (e.g. a pine crop followed by summer fallow with weed control and fall planting of hardwoods). Further investigation into nematode population response to hardwood crops are needed before implementing rotations with pine production systems, as unforeseen problems can occur (Cram and Fraedrich 2005)

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